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(71) Applicants *(for all designated States except US)*: **DYAX CORP.** [US/US]; 300 Technology Square, 8th Floor, Cambridge, MA 02139 (US). **BRACCO INTERNATIONAL B.V.** [NL/NL]; Stravinskylaan 3051, NL-1077 ZX Amsterdam (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SATO, Aaron, K.** [US/US]; 11 Pembroke Street, #2, Somerville, MA 02145 (US). **DRANSFIELD, Daniel, T.** [US/US]; 14 George Street, Hanson, MA 02341 (US). **LADNER, Robert, C.** [US/US]; 3827 Green Valley Road, Ijamsville, MD 21754 (US). **SHRIVASTAVA, Ajay** [IN/US]; 18-16 Pheasant Hollow Drive, Plainsboro, NJ 08536 (US). **NANJAPPAN, Palaniappa** [US/US]; 30 Liberty Drive, Dayton, NJ 08810 (US).

(74) Agent: **BUTLER LAWRENCE, Laurie**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

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PEPTIDES THAT SPECIFICALLY BIND HGF RECEPTOR (cMet) AND USES THEREOF

This application claims priority under 35 USC §119(e) to U.S. Provisional Patent Application Serial No.60/451,588, filed on March 3, 2003, the entire contents of which is hereby incorporated by reference.

Background of the Invention

Hepatocyte growth factor (also known as scatter factor) is a multi-functional growth factor involved in various physiological processes such as embryogenesis, wound healing and angiogenesis. It has become apparent that HGF, through interactions with its high affinity receptor (cMet), is involved in tumor growth, invasion and metastasis. In fact, dysregulated cMet expression (for example, the overexpression of cMet in neoplastic epithelium of colorectal adenomas and in other carcinomas as compared to normal mucosa) and/or activity, as well as hyperactivity of the cMet receptor through an autocrine stimulatory loop with HGF, has been demonstrated in a variety of tumor tissues and induces oncogenic transformation of specific cell lines.

In general, HGF is produced by the stromal cells, which form part of many epithelial tumors; however, it is believed that the production of HGF by tumor cells themselves comprises the main pathway leading to the hyperproliferation of specific tumors. HGF/cMet autocrine stimulatory loops have been detected in gliomas, osteosarcomas, and mammary, prostate, breast, lung and other carcinomas.

Interrupting the HGF interaction with the cMet receptor slows tumor progression in animal models. In addition to stimulating proliferation of certain cancer cells through activation of cMet, HGF also protects against DNA-damaging agent-induced cytotoxicity in a variety of cell lines susceptible to hyperproliferative phenotypes (*e.g.*, breast cancer). Therefore, preventing HGF from binding to cMet could predispose certain cancer cells to the cytotoxicity of certain drugs.

In addition to hyperproliferative disorders, cMet also has been linked to angiogenesis. For example, stimulation of cMet leads to the production of vascular

endothelial growth factor (VEGF), which, in turn, stimulates angiogenesis. Additionally, stimulation of cMet also has been implicated in promoting wound healing.

In addition to identifying the cMet receptor as a therapeutic target for hyperproliferative disorders, angiogenesis and wound healing, the large discrepancy between expression levels of neoplastic and corresponding normal tissues indicates that cMet is an attractive target for imaging applications directed to hyperproliferative disorders.

Summary of the Invention

The present invention relates to peptides, peptide complexes and compositions having the ability to bind to cMet and antagonize hepatocyte growth factor (HGF) activity by preventing HGF from binding to cMet. In addition, this invention relates to such peptides, peptide complexes and compositions having the ability to bind to cMet for the purpose of detecting and targeting this receptor, inhibiting cMet activity independent of HGF antagonistic properties, and for the purpose of diagnostic imaging. The involvement of the HGF/cMet axis in a variety of cellular functions including cellular proliferation, wound healing and angiogenesis, leading to hyperproliferative diseases such as cancer, make the present invention particularly useful for interrupting HGF-mediated physiological events, for targeting substances, *e.g.*, therapeutics, including radiotherapeutics, to such sites, and for imaging important sites of cellular hyperproliferation.

In answer to the need for improved materials and methods for detecting, localizing, imaging, measuring and possibly inhibiting or affecting, *e.g.*, hyperproliferation and/or angiogenesis, it has been surprisingly discovered that twelve classes of non-naturally occurring polypeptides bind specifically to cMet. Appropriate labeling of such polypeptides provides detectable imaging agents that can bind, *e.g.*, at high concentration, to cMet-expressing cells or cells exhibiting HGF/cMet complexes, providing specific imaging agents for sites of cellular proliferation and/or angiogenesis. The cMet binding polypeptides of the instant invention can thus be used in the detection and diagnosis of such hyperproliferative-related and/or angiogenesis-related disorders. Conjugation or fusion of such polypeptides with effective agents such as cMet

inhibitors or tumoricidal agents also can be used to treat pathogenic tumors, *e.g.*, by causing the conjugate or fusion to "home" to the site of active proliferation and/or angiogenesis, thereby providing an effective means for treating pathogenic conditions associated with hyperproliferation and/or angiogenesis.

This invention pertains to cMet binding polypeptides, and includes use of a single binding polypeptide as a monomer or in a multimeric or polymeric construct as well as use of more than one binding polypeptide of the invention in multimeric or polymeric constructs. Binding polypeptides according to this invention are useful in any application where binding, inhibiting, detecting or isolating cMet, or fragments thereof retaining the polypeptide binding site, is advantageous. A particularly important aspect of such binding polypeptides is the inhibition of cMet activity, either through competition with HGF for cMet binding, or by directly inhibiting cMet activity irrespective of whether HGF is bound or not. For example, in some cases, cMet signaling can occur in the absence of HGF binding, in such situations, a binding polypeptide that inhibits cMet signaling activity irrespective of whether HGF is bound, would be useful in inhibiting cMet signaling.

Another particularly advantageous use of the binding polypeptides disclosed herein is in a method of imaging cellular proliferation and/or angiogenesis *in vivo*. The method entails the use of specific binding polypeptides according to the invention for detecting a site of cellular proliferation and/or angiogenesis, where the binding polypeptides have been detectably labeled for use as imaging agents, including magnetic resonance imaging (MRI) contrast agents, x-ray imaging agents, radiopharmaceutical imaging agents, ultrasound imaging agents, and optical imaging agents.

Yet another advantageous use of the cMet binding polypeptides disclosed herein is to target therapeutic agents, (including compounds capable of providing a therapeutic, radiotherapeutic or cytotoxic effect) or delivery vehicles for therapeutics (including drugs, genetic material, etc.) to sites of hyperproliferation and/or angiogenesis or other tissue expressing cMet.

The cMet receptor is part of the receptor tyrosine kinase family of signaling molecules. For the purposes of the present invention, receptor tyrosine kinase function

can include any one of: oligomerization of the receptor, receptor phosphorylation, kinase activity of the receptor, recruitment of downstream signaling molecules, induction of genes, induction of cell proliferation, induction of cell migration, or combination thereof. "Heteromeric" molecules, used herein to refer to molecules containing more than one cMet binding peptide as described herein, such that each binding peptide of the heteromeric molecule binds to a different site, *e.g.*, "epitope", of cMet, also are encompassed by the present invention. For example, heteromeric constructs of binding polypeptides provided herein could, for example, bind, via one binding peptide, to, for example, the HGF binding site of cMet, while another binding peptide of the heteromeric molecule binds to a different high affinity binding site of cMet. Targeting two or more distinct epitopes on cMet with a single binding construct can greatly improve the ability of the construct to inhibit HGF binding and/or receptor function (such inhibition can occur by direct inhibition of cMet irrespective of HGF binding). Even binding peptides with weak ability to block receptor activity can be used to generate heteromeric constructs having improved ability to block HGF-dependent and HGF-independent receptor function.

Therefore, the present invention is drawn to constructs comprising means for producing multimeric molecules comprising two or more binding polypeptides, at least one of which binds cMet. In one embodiment, the multimeric constructs comprise two or more copies of a single binding polypeptide or nucleotide sequence that encode two or more copies of a single binding polypeptide. In another embodiment, the multimeric constructs of the present invention comprise two or more binding polypeptides or nucleotide sequence that encode two or more binding polypeptides, such that at least two of the binding polypeptides in the construct are specific for different epitopes of cMet. These constructs also are referred to herein as "heteromeric constructs", "heteromultimers", etc. The constructs of the present invention also can include unrelated, or control peptide. The constructs can include two or more, three or more, or four or more binding polypeptides or the nucleotide sequences that encode such polypeptides. Based on the teachings provided herein, one of ordinary skill in the art is able to assemble the binding polypeptides provided herein into multimeric constructs and to select multimeric constructs having improved properties, such as improved

ability to bind the target molecule, or improved ability to inhibit receptor tyrosine kinase function. Such multimeric constructs having improved properties are included in the present invention.

Consensus sequences from the screen of the cyclic/linear peptide libraries have been determined based on the twelve classes of specific cMet binding polypeptides shown in Table 6. In specific embodiments, cMet binding polypeptides of the invention comprise one or more of these sequences. Such preferred cMet binding polypeptides include polypeptides with the potential to form a cyclic or loop structure between invariant cysteine residues comprising.

The polypeptides described herein can have additional amino acids attached at either or both of the – and C-terminal ends. In preferred embodiments, binding polypeptides according to the invention can be prepared having N-terminal and/or C-terminal flanking peptides of one or more, preferably two, amino acids corresponding to the flanking peptides of the display construct of the phage selectant from which the binding polypeptides were isolated. Preferred N-terminal flanking peptides include Gly-Ser- (most preferably for TN6 sequences), Ala-Gly- (most preferably for TN8 and TN9 sequences), Gly-Ser- (most preferably for TN10 and TN11 sequences), Gly-Asp- (most preferably for TN12 sequences), Ala-Gln- (most preferably for linear sequences). Preferred C-terminal flanking peptides include -Ala-Pro (most preferably for TN6 sequences), -Gly-Thr (most preferably for TN8 and TN9 sequences), -Ala-Pro (most preferably for TN10 and TN11 sequences), -Asp-Pro (most preferably for TN12 sequences), -Asp-Phe (most preferably for linear sequences). Single terminal amino acids also can be added to the binding polypeptides of the invention, and preferred terminal amino acids will correspond to the parental phage display construct, *e.g.*, most preferably, N-terminal amino acids will be selected from Gly- (most preferably for TN6, TN8 and TN9 sequences), Ser- (most preferably for TN10 and TN11 sequences), Asp- (most preferably for TN12 sequences), and Gln- (most preferably for linear sequences), and most preferably C-terminal amino acids will be selected from -Gly (most preferably for TN6, TN8 and TN9, and linear sequences), -Ala (most preferably for TN10 and TN11 sequences), and -Asp (most preferably for TN12 sequences). Conservative substitutions (*i.e.*, substitute amino acids selected within the following

groups: {Arg, His, Lys}, {Glu, Asp}, {Asn, Cys, Glu, Gly, Ser, Thr, Tyr}, {Ala, Ile, Leu, Met, Phe, Pro, Trp, Val}) for such flanking amino acids also are contemplated.

Examination of the sequence information and binding data from the isolates of libraries containing polypeptides with the potential to form loop structures (*e.g.*, libraries designated TN6, TN8, TN9, TN10, TN11 and TN12; the number refers to the number of amino acids in the sequence from cysteine to cysteine; additionally, the linear display library, LN20, also was screened) identifies an additional series of cMet binding polypeptides. A consensus motif was obtained from this initial screen of a TN9 library (CxGpPxFxC; SEQ ID NO:512). The consensus sequence was derived from the sequences listed in Table 6. This consensus sequence along with sequence trends in the cMet binding peptides identified from the linear peptide library was used to design a second generation library that was used in a secondary screen. Sequences from both screens were used to identify twelve classes of cMet binding motifs listed in Table 6.

Another aspect of the present invention relates to modifications of the polypeptides of the invention to provide specific cellular proliferation and/or angiogenesis imaging agents by detectably labeling a polypeptide or multimeric polypeptide construct according to the present invention. Such detectable labeling can involve radiolabeling, enzymatic labeling, or labeling with MR paramagnetic chelates or microparticles; incorporation into ultrasound bubbles, microparticles, microspheres, emulsions, or liposomes; or conjugation with optical dyes.

In another aspect of the present invention, methods for isolating cMet-expressing cells using the present binding polypeptides or multimeric polypeptide construct are provided.

Additionally, the cMet binding polypeptides or multimeric polypeptide construct of the invention can be used as therapeutic agents, either alone in a pharmaceutically acceptable composition or conjugated to (or in combination with) other therapeutic agents. The compositions can be used to treat diseases or conditions involving cellular proliferation, angiogenesis and/or wound healing.

When used as therapeutic agents, it may be advantageous to enhance the serum residence time of the peptides. This can be accomplished by: a) conjugating to the peptide a moiety, such as maleimide, that reacts with free sulfhydryl groups on serum

proteins, such as serum albumin, b) conjugating to the peptide a moiety, such as a fatty acid, that binds non-covalently to serum proteins, especially serum albumin, c) conjugating to the peptide a polymer, such as polyethylene glycol (PEG), that is known to enhance serum residence time, and d) fusing DNA that encodes the cMet-binding peptide to DNA that encodes a serum protein such as human serum albumin or an antibody and expressing the encoded fusion protein.

In another aspect of the invention, methods of screening polypeptides identified by phage display for their ability to bind to cells expressing the target are provided. These methods permit rapid screening of the binding ability of polypeptides, including polypeptides with monomeric affinities that are too low for evaluation in standard cell-binding assays. Additionally, these methods can be used to rapidly assess the stability of the peptides in the presence of serum.

In one embodiment, the present invention is directed to a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid. In a particular embodiment, X_2 is Pro.

In another embodiment, the polypeptides of the invention further comprises N-terminal and/or C-terminal flanking peptides of one or more amino acids. For example, the polypeptide can comprise a modification selected from the group consisting of: an amino acid substitution, and amide bond substitution, a D-amino acid substitution, a glycosylated amino acid, a disulfide mimetic substitution, an amino acid translocation, a retro-inverso peptide, a peptoid, a retro-inverso peptoid and a synthetic peptide. In another embodiment, any of the polypeptides described herein can be conjugated to a detectable label or a therapeutic agent, optionally further comprising a linker or spacer between the polypeptide and the detectable label or the therapeutic agent. In a particular embodiment, the detectable label or the therapeutic agent is selected from the group consisting of: an enzyme, a fluorescent compound, a liposome, an optical dye, a paramagnetic metal ion, an ultrasound contrast agent and a radionuclide. In a particular embodiment, the therapeutic agent or detectable label comprises a radionuclide. For example, the radionuclide can be one ore more selected from the group consisting of:

^{18}F, ^{124}I, ^{125}I, ^{131}I, ^{123}I, ^{77}Br, ^{76}Br, ^{99m}Tc, ^{51}Cr, ^{67}Ga, ^{68}Ga, ^{47}Sc, ^{51}Cr, ^{167}Tm, ^{141}Ce, ^{111}In, ^{168}Yb, ^{175}Yb, ^{140}La, ^{90}Y, ^{88}Y, ^{153}Sm, ^{166}Ho, ^{165}Dy, ^{166}Dy, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{97}Ru, ^{103}Ru, ^{186}Re, ^{188}Re, ^{203}Pb, ^{211}Bi, ^{212}Bi, ^{213}Bi, ^{214}Bi, ^{105}Rh, ^{109}Pd, ^{117m}Sn, ^{149}Pm, ^{161}Tb, ^{177}Lu, ^{198}Au and ^{199}Au. In another embodiment, the therapeutic agent or detectable label further comprises a chelator. For example, the chelator can comprise a compound selected from the group consisting of: formula 20, 21, 22, 23a, 23b, 24a, 24b and 25. In a particular embodiment, the radionuclide is ^{99m}Tc or ^{111}In. In another embodiment, the radionuclide is selected from the group consisting of: ^{177}Lu, ^{90}Y, ^{153}Sm and ^{166}Ho. In another embodiment, the detectable label comprises an ultrasound contrast agent. For example, the ultrasound contrast agent can comprise a phospholipid stabilized microbubble or a microballoon comprising a gas, *e.g.*, a fluorinated gas. In another embodiment, the detectable label comprises a paramagnetic metal ion and a chelator. Another aspect of the invention is directed to any of the polypeptides of the invention, wherein the therapeutic agent is selected from the group consisting of: a bioactive agent, a cytotoxic agent, a drug, a chemotherapeutic agent or a radiotherapeutic agent. In other embodiments, the polypeptide has an apparent K_D for cMet of cMet/HGF complex of less than about 10μM, less than about 1.0μM, less than about 0.1μM or less than about 1nM.

In one embodiment, the present invention is directed to a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence of one of the following classes: Class 1: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7-Cys-X_8-X_9-X_{10} (TN6), wherein X_1 is Phe, Leu, Ser, Trp, Tyr or Met; X_2 is Ile, Tyr, His, Thr or Asn; X_3 is Ile, Leu, Asp, Met, Phe or Ser; X_4 is Arg, Asn, Glu, Pro or Trp; X_5 is Glu, Gly, Leu, Pro, Thr, Trp or Tyr; X_6 is Asp, Gln, Glu Gly, Phe, Ser, Thr or Trp; X_7 is Ala, Arg, Asn, Gln, Glu, Gly, Phe, or Trp; X_8 is Gly, Asn, His, Arg, Met, Ile, Asp, Val or Thr; X_9 is Ser, Lys, Phe, Met, Thr, Asp or Leu; and X_{10} is Ser, Pro, Thr, Leu, Tyr, Asn, His, Glu or Trp; or Class II: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7-X_8-X_9-Cys-X_{10}-X_{11}-X_{12} (TN8), wherein X_1 is Gly, Val, Trp, Thr, Lys or Gln; X_2 is Trp, Tyr, Leu, Phe or Thr; X_3 is Trp, Glu, Phe, Ile, Leu and Ser; X_4 is Asn, Gln or Glu; X_5 is Leu, Glu or Trp; X_6 is Glu, Ser or Tyr; X_7 is Glu, Met or Pro; X_8 is Met, Ser or Trp; X_9 is Leu, Phe or Val; X_{10} is Asp, Glu or Trp; X_{11} is Met, Phe or

Trp; and X_{12} is Gln, Leu or Trp; or Class III: X_1-X_2-X_3-Cys-X_4-Gly-X_5-Pro-X_6-Phe-X_7-Cys-X_8-X_9 (TN9), wherein X_1 is Glu, Ser, Trp or Tyr; X_2 is Phe, Thr or Trp; X_3 is His, Phe or Trp; X_4 is Ala, Lys, Ser or Thr; X_5 is Pro or Trp; X_6 is Ser or Thr; X_7 is Glu or Ser; X_8 is Ile, Trp or Tyr; and X_9 is Glu, Met, Trp or Tyr; or Class IV-1: X_1-X_2-X_3-Cys-X_4-Gly-Pro-Pro-X_5-Phe-X_6-Cys-Trp-X_7-X_8-X_9-X_{10}-X_{11} (TN9), wherein X_1 is Arg, Asp, Asn, Ile or Ser; X_2 is Leu, Ile, Phe, Trp or Val; X_3 is Asn, Gln, His, Leu, Tyr or Val; X_4 is Leu, Lys or Ser; X_5 is Ala, Ser, Thr or Trp; X_6 is Leu, Ser or Trp; X_7 is Leu, Ser or Trp; X_8 is Phe or Tyr; X_9 is Asp, Glu, Gly or Val; X_{10} is Met, Pro, Thr or Ser; and X_{11} is Glu or Gly; or Class IV-2: X_1-X_2-X_3-X_4-Trp-X_5-Cys-X_6-Gly-Pro-Pro-Thr-Phe-Glu-Cys-Trp-X_7-X_8 (TN9), wherein X_1 is Asp, Glu or Val; X_2 is Ala, Asp, Gly, Ser or Val; X_3 is Asp, Gly, Ser or Val; X_4 is Arg, Asn, Gly, Ser or Thr; X_5 is Gln or His; X_6 is Asn, Lys or Ser; X_7 is Ser or Trp; and X_8 is Phe or Tyr; or Class V: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-Cys-X_{12}-X_{13}-X_{14} (TN10), wherein X_1 is His, Phe, Pro, Thr or Trp; X_2 is Ala, Arg, Glu, His, Lys or Phe; X_3 is Met, Phe, Pro, Thr or Val; X_4 is His, Leu, Met, Phe or Trp; X_5 is Arg, Asp, Glu, Gly, Met or Trp; X_6 is Glu, Gly, Ile, Lys, Phe or Pro; X_7 is Asp, Phe, Pro, Ser, Trp or Tyr; X_8 is Ala, Arg, Asn, Phe or Ser; X_9 is Ala, Gln, Gly, Leu or Phe; X_{10} is Gln, Gly, Ile, Leu, Trp or Tyr; X_{11} is Arg, Asp, Phe, Pro, Tyr or Val; X_{12} is Asn, Gln, His, Ile or Thr; X_{13} is Ala, Asn, Asp, Glu or His; and X_{14} is Asn, Gln, Glu, His or Val; or Class VI: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-Cys-X_{13}-X_{14}-X_{15}, wherein X_1 is Gln, Gly, Met, Phe or Ser; X_2 is Asn, Gln, Leu or Met; X_3 is Arg, Asn, Gly, His or Ile; X_4 is Asn, Asp, Leu, Thr or Trp; X_5 is Arg, Gln, Thr, Tyr or Val; X_6 is Glu, Gly, Leu, Met or Thr; X_7 is Ala, Asn, Asp, His, Ile, Leu or Ser; X_8 is Arg, Gln, Ser, Thr or Tyr; X_9 is Asp, Gly, Ile or Phe; X_{10} is Gln, Phe or Thr; X_{11} is Gln, His, Phe, Pro, Ser or Tyr; X_{12} is Asn, Asp, Phe, Pro or Ser; X_{13} is Ala, Asn, Gly, Leu or Ser; X_{14} is Arg, Pro, Ser or Val; and X_{15} is Asp, Glu, Leu or Met; or Class VIII: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-Cys-X_{14}-X_{15}-X_{16}, wherein X_1 is Ala, His, Leu, Phe or Tyr; X_2 is Arg, Asp, Leu, Ser or Tyr; X_3 is Glu, Met or Trp; X_4 is Asp, Gln, Glu, Phe or Ser; X_5 is Glu, Ile, Phe or Trp; X_6 is Asn, Asp or Ser; X_7 is Asn, Asp or Leu; X_8 is Asp, Glue or Lys; X_9 is Gly, Phe or Thr; X_{10} is Gly, Phe, Trp or Tyr; X_{11} is Glu, Ser or Trp; X_{12} is Glu, Phe, Tyr or Val; X_{13} is Glu, Lys, Thr or Val; X_{14} is Glu or Trp; X_{15} is Asp, Phe, Pro, Ser or Trp; and X_{16} is Ala, Asn or Ile; or Class IX-1: Ser-Cys-

X_1-Cys-X_2-Gly-Pro-Pro-Thr-Phe-Glu-Cys-Trp-Cys-Tyr-X_3-X_4-X_5, wherein X_1 is Asn, His or Tyr; X_2 is Gly or Ser; X_3 is Ala, Asp, Glu, Gly or Ser; X_4 is Ser or Thr; and X_5 is Asp or Glu; or Class IX-2: Glu-X_1-Gly-Ser-Cys-His-Cys-Ser-Gly-Pro-Pro-Thr-Phe-Glu-Cys-X_2-Cys-X_3, wherein X_1 is Ala, Glu, Gly or Ser; X_2 is Phe, Trp or Tyr; and X_3 is Phe or Tyr.

In another embodiment, the invention is directed to a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence, wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511. In a particular embodiment, the polypeptide, used as either a monomer or in a multimeric construct, can be selected from the group consisting of SEQ ID NOS:1-511, SEQ ID NOS:1-10, SEQ ID NOS:11-47, SEQ ID NOS:48-101, SEQ ID NOS:102-364, SEQ ID NOS:365-370, SEQ ID NOS:371-387, SEQ ID NO:388 or SEQ ID NO:399, SEQ ID NOS:390-404, SEQ ID NOS:405-447, SEQ ID NO:448, SEQ ID NOS:449-496 and SEQ ID NOS:497-511.

In another embodiment, the invention is directed to a method for isolating phage that bind cMet or a complex comprising cMet and HGF, comprising the steps of: immobilizing cMet or a complex comprising cMet and HGF on a solid support; contacting a library of potential cMet or cMet/HGF complex binding phage with the solid support to bind cMet or cMet/HGF binding phage in the library; and removing the unbound portion of the phage library from the solid support, thereby isolating phage that bind cMet or a complex comprising cMet and HGF.

In another embodiment, the invention is directed to a method of detecting cMet or a complex comprising cMet and HGF in an animal or human subject and optionally imaging at least a portion of the animal or human subject comprising the steps of: detectably labeling a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid; administering to the subject the labeled polypeptide or multimeric polypeptide construct; and, detecting the labeled polypeptide or construct in

the subject, and, optionally, constructing an image, thereby detecting cMet or a complex comprising cMet and HGF.

In a particular embodiments, the methods of the invention encompass methods wherein the label is selected from the group consisting of: an enzyme, a fluorescent compound, an ultrasound contrast agent, a liposome and an optical dye, wherein the label optionally further comprises a linker and/or a spacer. In particular embodiment, the ultrasound contrast agent is a phospholipid stabilized microbubble or a microballoon comprising a gas, *e.g.*, a fluorinated gas. In other embodiments, the label is a radioactive label or a paramagnetic metal atom, and optionally further comprises a linker or a spacer. In another embodiment, the radioactive label comprises a radionuclide selected from the group consisting of: ^{18}F, ^{124}I, ^{125}I, ^{131}I, ^{123}I, ^{77}Br, ^{76}Br, ^{99m}Tc, ^{51}Cr, ^{67}Ga, ^{68}Ga, ^{47}Sc, ^{51}Cr, ^{167}Tm, ^{141}Ce, ^{111}In, ^{168}Yb, ^{175}Yb, ^{140}La, ^{90}Y, ^{88}Y, ^{153}Sm, ^{166}Ho, ^{165}Dy, ^{166}Dy, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{97}Ru, ^{103}Ru, ^{186}Re, ^{188}Re, ^{203}Pb, ^{211}Bi, ^{212}Bi, ^{213}Bi, ^{214}Bi, ^{105}Rh, ^{109}Pd, ^{117m}Sn, ^{149}Pm, ^{161}Tb, ^{177}Lu, ^{198}Au and ^{199}Au. In another embodiment, the radioactive label further comprises a chelator, *e.g.*, chelators selected from the group consisting of: formula 20, 21, 22, 23a, 23b, 24a, 24b and 25. In another embodiment, the radionuclide is ^{99m}Tc or ^{111}In. In a particular embodiment, the paramagnetic label comprises a paramagnetic metal atom selected from the group consisting of: Mn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Gd^{3+}, Eu^{3+}, Dy^{3+}, Pr^{3+}, Cr^{3+}, Co^{3+}, Fe^{3+}, Ti^{3+}, Tb^{3+}, Nd^{3+}, Sm^{3+}, Ho^{3+}, Er^{3+}, Pa^{4+} and Eu^{2+}. In another embodiment, the paramagnetic label further comprises a chelator, *e.g.*, a chelator is selected from the group consisting of: DTPA, DO3A, DOTA, EDTA, TETA, EHPG, HBED, NOTA, DOTMA, TETMA, PDTA, TTHA, LICAM, and MECAM. In particular embodiments, detection of the labeled polypeptide or multimeric polypeptide construct is indicative of a hyperproliferative disorder. In other embodiments, detection of the labeled polypeptide or multimeric polypeptide construct is indicative of angiogenesis or neovascularization. In particular embodiments, the label is an ultrasound contrast agent that comprises a fluorinated gas selected from the group of: SF_6 freons, CF_4, C_2F_6, C_3F_8, C_4F_{10}, $CBrF_3$, CCl_2F_2, C_2ClF_5, $CBrClF_2$ and perfluorocarbons. In particular embodiments, the ultrasound contrast agent comprises a perfluorocarbon gas having the formula C_nF_{n+2} wherein n is from 1 to 12.

In another embodiment, the invention is directed to a method of detecting cMet or a complex comprising cMet and HGF in an animal or human subject and optionally imaging at least a portion of the animal or human subject comprising the steps of: detectably labeling a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence, wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511; administering to the subject the labeled polypeptide or construct; and, detecting the labeled polypeptide or construct in the subject, and, optionally, constructing an image, thereby detecting cMet or a complex comprising cMet and HGF.

In another embodiment, the invention is directed to a method of treating a condition involving activation of cMet, comprising administering to an animal or human subject in need of treatment for such a condition a composition comprising a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid. In another embodiment, the invention is directed to a method of treating a condition involving activation of cMet, comprising administering to an animal or human subject in need of treatment for such a condition a composition comprising a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence, wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511. In a particular embodiment, the condition is solid tumor growth, *e.g.*, wherein the tumor is selected from the group consisting of breast, thyroid, glioblastoma, prostate, malignant mesothelioma, colorectal, hepatocellular, hepatobiliary, renal, osteosarcoma and cervical. In a particular embodiment, the polypeptide or multimeric polypeptide construct can be conjugated to a tumoricidal agent.

In another embodiment, the invention is directed to a recombinant bacteriophage displaying any one or more of the polypeptides or multimeric polypeptide construct

described herein or having any one or more of the consensus sequences described herein, such that the phage has the ability to bind to cMet or a complex comprising cMet and HGF, and wherein the polypeptide is displayed on the surface of the recombinant bacteriophage.

In another embodiment, the invention is directed to a magnetic resonance imaging contrast agent comprising a composition comprising a polypeptide having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid, or wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511. In a particular embodiment, the magnetic resonance imaging contrast agent further comprises at least one paramagnetic metal atom, *e.g.*, at least one chelator selected from the group consisting of: DTPA, DOTA, EDTA, TETA, EHPG, HBED, NOTA, DOTMA, TETMA, PDTA, TTHA, LICAM, and MECAM. In particular embodiments, the chelator is selected from the group consisting of: diethylenetriamine, tetraazacyclododecane and a carboxymethyl-substituted derivative thereof. In other embodiments, the paramagnetic metal atom is selected from the group consisting of: Mn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Gd^{3+}, Eu^{3+}, Dy^{3+}, Pr^{3+}, Cr^{3+}, Co^{3+}, Fe^{3+}, Ti^{3+}, Tb^{3+}, Nd^{3+}, Sm^{3+}, Ho^{3+}, Er^{3+}, Pa^{4+} and Eu^{2+}. In a particular embodiment, the multivalent cation is Gd^{3+}.

In another embodiment, the invention is directed to a method for identifying cMet or cMet/HGF complex binding compounds comprising the steps of: utilizing a cMet or cMet/HGF complex binding polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid, to form a complex with a cMet or cMet/HGF complex target; contacting the complex with one or more potential cMet or cMet/HGF complex binding compounds; and determining whether the potential cMet or cMet/HGF complex binding compound competes with the cMet or cMet/HGF complex binding polypeptide to form a complex with the cMet or cMet/HGF complex target.

In one embodiment, the invention is directed to a diagnostic imaging contrast agent comprising a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid, or wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511.

In another embodiment, the invention is directed to a method of medical imaging comprising the steps of administering to an animal or human subject a pharmaceutical preparation of a contrast agent comprising at least one polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid, and imaging the contrast agent by a method selected from the group consisting of: magnetic resonance imaging, ultrasound imaging, optical imaging, sonoluminescence imaging, photoacoustic imaging, and nuclear imaging. In another embodiment, the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511, and imaging the contrast agent by a method selected from the group consisting of: magnetic resonance imaging, ultrasound imaging, optical imaging, sonoluminescence imaging, photoacoustic imaging, and nuclear imaging.

In another embodiment, the invention is directed to a method of radiotherapy comprising administering to an animal or human subject in need of such therapy a compound comprising at least one polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid, or wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511, conjugated to a radionuclide useful for radiotherapy. In a particular embodiment, the compound further comprises a chelator, *e.g.*, a compound selected from the group consisting of: formula 20, 21, 22,

23a, 23b, 24a, 24b and 25. In another embodiment, the compound further comprises a spacer or linker. In a particular embodiment, the radionuclide can be ^{186}Re, ^{188}Re, ^{177}Lu, ^{90}Y, ^{153}Sm or ^{166}Ho.

In another embodiment, the invention is directed to a kit for preparation of a radiopharmaceutical comprising a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid, or wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511, a chelator for a radionuclide, and a reducing agent.

In another embodiment, the invention is directed to a method of targeting genetic material to cMet-expressing cells comprising administering to an animal or a human in need of such genetic material a polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF comprising an amino acid sequence comprising Cys-X_1-Gly-X_2-Pro-X_3-Phe-X_4-Cys, wherein X_1, X_2, X_3 and X_4 can be any amino acid, or wherein the amino acid sequence comprises at least six amino acids out of a contiguous stretch of nine amino acids from a sequence selected from the group consisting of SEQ ID NOS:1-511, conjugated to or associated with the genetic material or a delivery vehicle containing such genetic material.

In another embodiment, the invention is directed to a method of screening binding polypeptides identified by phage display for their ability to bind to cells expressing the cMet or cMet/HGF target comprising the steps of preparing multimeric constructs including one or more binding polypeptides; contacting the multimeric constructs with cells expressing the target and assessing the ability of the multimeric constructs to bind to the target. In a particular embodiment, the cells can be engineered by recombinant DNA technology to express the target. In another embodiment, the multimeric constructs can be detectably labeled. In another embodiment, the ability of the multimeric constructs to bind to the target is assessed in the presence of serum. In

another embodiment, the multimeric construct can comprise biotinylated binding polypeptides complexed with avidin, streptavidin or neutravidin.

Brief Description of the Drawings

FIGS. 1A-1C are representations of mimics, which can be employed to mimic structural motifs and turn features in a peptide and simultaneously provide stability to proteolysis and enhance other properties (structure 1A: Hart, S. and Etzkorn, F., 1999. *J. Org. Chem.*, 64:2998-2999; structure 1B: Hanessian, S. and McNaughton-Smith, G., "Synthesis of a Versatile Peptidomimetic Scaffold" in *Methods in Molecular Medicine, Vol. 23: Peptidomimetics Protocols*, W. Kazmierski, Ed. (Humana Press Inc., Totowa, N.J., 1999), Chapter 10, pp. 161-174; structure 1C: WO 01/16135.

FIG. 2 is a representation of the amino acids (4), containing an aminoalcohol function, and (5) containing an alkoxyamino function.

FIG. 3 is a representation depicting the cyclization of Cysteine with a pendant bromoacetamide function (this process is referred to herein as "scheme 1").

FIG. 4 is a representation showing intramolecular cyclization of suitably located vicinal amino mercaptan functions and aldehyde functions to provide thiazolidines that result in the formation of a bicyclic peptide, one ring of which is that formed by the residues in the main chain, and the second ring being the thiazolidine ring (this process is referred to herein as "scheme 2").

FIG. 5 is a representation showing how a lactam function, available by intramolecular coupling via standard peptide coupling reagents (such as HATU, PyBOP etc) can act as a surrogate for the disulfide bond. The Dde/Dmab approach is shown (and is referred to herein as "scheme 3").

FIG. 6 is a representation showing the Grubbs reaction (referred to herein as "scheme 4").

FIGS. 7A and 7B are chemical structures of phospholipid moieties.

FIGS. 8A-F depict structures of preferred metal chelators.

FIG. 9 is a schematic representation of the selection strategy that was employed to identify cMet binding polypeptides. TEA = triethylamine, Bead Infection = capture of non-eluted phage that remained bound to the cMet-Fc/protein-A beads.

FIG. 10 illustrates the growth inhibitory properties of cMet-binding peptide SEQ ID NO:365.

FIG. 11 shows a schematic diagram for the preparation of SEQ ID NO:514 conjugated to a 6-PnAO-Glut moiety, (referred to herein as "scheme 5").

FIG. 12 shows a schematic diagram for the preparation of a heterodimer containing SEQ ID NOS: 514 and 515 joined by a K(PnAO6-Glut) linker (referred to herein as "scheme 5").

FIGS. 13A-13C show the chemical structures of three heterodimers as follows: FIG. 13A shows SEQ ID NO:514 linked to SEQ ID NO:515 (Ac-GSPEMCMMFPFLYPCNHHAPGGGK{PnAO6-Glut-K[Ac-GSFFPCWRIDRFGYCHANAPGGGKJJ-Glut]-NH2}-NH2); FIG. 13B shows SEQ ID NO:515 linked to SEQ ID NO:516 (Ac-GSFFPCWRIDRFGYCHANAPGGGK{PnAO6-Glut-K[Ac-AQEWEREYFVDGFWGSWFGIPHGGGK(JJ-Glut)-NH2]}-NH2); and FIG. 13C shows SEQ ID NO:514 linked to SEQ ID NO:517 (Ac-GSPEMCMMFPFLYPCNHHAPGGGK{PnAO6-Glut-K[Ac-GDYSECFFEPDSFEVKCYDRDPGGGK(JJ-Glut)-NH2]}-NH2).

FIG. 14 is a graphical representation of data showing binding of derivatives of SEQ ID NO:514 with different spacer length and biotin. Derivatives have none, one J and two J spacers respectively in between the targeting sequence and biotin.

Detailed Description of the Invention

A description of preferred embodiments of the invention follows.

The present invention provides novel binding moieties that bind to the hepatocyte growth factor receptor ("HGFr" or "cMet"). Such binding moieties make possible the efficient detection, imaging and localization of activated cells exhibiting upregulated cMet expression and binding of HGF to cMet. Such activated cells are initiators of cellular proliferation, and therefore the polypeptides described herein provide a means of detecting, monitoring and localizing sites of proliferation. In particular, the binding moieties of this invention, which include polypeptides and multimeric polypeptide constructs, when appropriately labeled, are useful for detecting,

imaging and localizing tumors or other proliferative disorders that result from dysregulated cellular proliferation (*e.g.*, cancer). Thus, the binding polypeptides and multimeric polypeptide constructs of the invention can be used to form a variety of diagnostic and therapeutic agents for diagnosing and treating neoplastic tumor growth or other proliferative disorders. In addition, the binding polypeptides and multimeric polypeptide constructs can themselves be used as therapeutic agents.

Specific cMet binding polypeptides according to the present invention were isolated initially by screening of phage display libraries, that is, populations of recombinant bacteriophage transformed to express an exogenous peptide on their surface. In order to isolate new polypeptide binding moieties for a particular target, such as cMet, screening of large peptide libraries, for example using phage display techniques, is especially advantageous, in that very large numbers (*e.g.*, 5×10^9) of potential binders can be tested and successful binders isolated in a short period of time.

In order to prepare a phage library of displaying polypeptides to screen for binding polypeptides such as cMet binding polypeptides and/or polypeptides that bind to a complex comprising HGF bound to cMet, a candidate binding domain is selected to serve as a structural template for the peptides to be displayed in the library. The phage library is made up of a multiplicity of analogues of the parental domain or template. The binding domain template can be a naturally occurring or synthetic protein, or a region or domain of a protein. The binding domain template can be selected based on knowledge of a known interaction between the binding domain template and the binding target, but this is not critical. In fact, it is not essential for the selected domain to act as a template for the library or have any affinity for the target at all; its purpose is to provide a structure from which a multiplicity (library) of similarly structured polypeptides (analogues) can be generated, which multiplicity of analogs will include one or more analogs that exhibit the desired binding properties (and any other properties screened for).

In selecting the parental binding domain or template on which to base the variegated amino acid sequences of the library, an important consideration is how the variegated peptide domains will be presented to the target, *i.e.*, in what conformation the peptide analogues will come into contact with the target. In phage display

methodologies, for example, the analogs are generated by insertion of synthetic DNA encoding the analogs into phage, resulting in display of the analog on the surfaces of the phage. Such libraries of phage, such as M13 phage, displaying a wide variety of different polypeptides, can be prepared using techniques as described, *e.g.*, in Kay *et al.*, *Phage Display of Peptides and Proteins: A Laboratory Manual* (Academic Press, Inc., San Diego, 1996) and US 5,223,409 (Ladner *et al.*), incorporated herein by reference.

In isolating the specific polypeptides according to this invention, seven cyclic peptide (or "loop") libraries, designated TN6, TN7, TN8, TN9, TN10, TN11, TN12, and a linear library, designated LN20, were initially screened. Each library was constructed for expression of diversified polypeptides on M13 phage. The seven libraries having a "TN" designation were designed to display a short, variegated exogenous peptide loop of 6, 7, 8, 9, 10, 11 or 12 amino acids, respectively, on the surface of M13 phage, at the amino terminus of protein III. The libraries are designated TN6 (having a potential 3.3×10^{12} amino acid sequence diversity), TN7 (having a potential 1.2×10^{14} amino acid sequence diversity), TN8 (having a potential 2.2×10^{15} amino acid sequence diversity), TN9 (having a potential 4.2×10^{16} amino acid sequence diversity, TN10 (having a potential 3.0×10^{16} amino acid sequence diversity), TN11 (having a potential 1.5×10^{19} amino acid sequence diversity), TN12 (having a sequence diversity of 4.6×10^{19}), and LN20 (having a potential 3.8×10^{25} amino acid sequence diversity).

The TN6 library was constructed to display a single microprotein binding loop contained in a 12-amino acid template. The TN6 library utilized a template sequence of Xaa1 - Xaa2 - Xaa3 - Cys - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Cys - Xaa10 - Xaa11 - Xaa12. The amino acids at positions 2, 3, 5, 6, 7, 8, 10, and 11 of the template were varied to permit any amino acid except cysteine (Cys). The amino acids at positions 1 and 12 of the template were varied to permit any amino acid except cysteine (Cys), glutamic acid (Glu), isoleucine (Ile), Lysine (Lys), methionine (Met), and threonine (Thr).

The TN7 library was constructed to display a single microprotein binding loop contained in a 13-amino acid template. The TN7 library utilized a template sequence of Xaa1 - Xaa2 - Xaa3 - Cys - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Xaa9 - Cys - Xaa11 - Xaa12 -

Xaa13. The amino acids at amino acid positions 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, and 13 of the template were varied to permit any amino acid except cysteine (Cys).

The TN8 library was constructed to display a single microprotein binding loop contained in a 14-amino acid template. The TN8 library utilized a template sequence of Xaa1 - Xaa2 - Xaa3 - Cys - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Xaa9 - Xaa10 - Cys - Xaa12 - Xaa13 - Xaa14. The amino acids at position 1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, and 14 in the template were varied to permit any amino acid except cysteine (Cys).

The TN9 library was constructed to display a single microprotein binding loop contained in a 15-amino acid template. The TN9 library utilized a template sequence Xaa1 - Xaa2 - Xaa3 - Cys - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Xaa9 - Xaa10 - Xaa11 - Cys - Xaa13 - Xaa14 - Xaa15. The amino acids at position 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 13, 14 and 15 in the template were varied to permit any amino acid except cysteine (Cys).

The TN10 library was constructed to display a single microprotein binding loop contained in a 16-amino acid template. The TN10 library utilized a template sequence Xaa1 - Xaa2 - Xaa3 - Cys - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Xaa9 - Xaa10 - Xaa11 - Xaa12 - Cys - Xaa14 - Xaa15 - Xaa16. The amino acids at positions 1, 2, 15, and 16 in the template were varied to permit any amino acid selected from a group of 10 amino acids: D, F, H, L, N, P, R, S, W, or Y). The amino acids at positions 3 and 14 in the template were varied to permit any amino acid selected from a group of 14 amino acids: A, D, F, G, H, L, N, P, Q, R, S, V, W, or Y). The amino acids at positions 5, 6, 7, 8, 9, 10, 11, and 12 in the template were varied to permit any amino acid except cysteine (Cys).

The TN11 library was constructed to display a single microprotein binding loop contained in a 17-amino acid template. The TN11 library utilized a template sequence Xaa1 -Xaa2 - Xaa3 - Cys - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Xaa9 - Xaa10 - Xaa11 - Xaa12 - Xaa13-Cys -Xaa15 - Xaa16 - Xaa17. The amino acids at positions 1 through 3, 5 through 13, and 15 through 17 in the template were varied to permit any amino acid except cysteine (Cys).

The TN12 library was constructed to display a single microprotein binding loop contained in an 18-amino acid template. The TN12 library utilized a template sequence Xaa1 - Xaa2 - Xaa3 - Cys - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Xaa9 - Xaa10 - Xaa11 -

Xaa12 - Xaa13 - Xaa14 - Cys - Xaa16 - Xaa17 - Xaa18. The amino acids at position 1, 2, 17, and 18 in the template were varied to permit any amino acid selected from a group of 12 amino acids: A, D, F, G, H, L, N, P, R, S, W, or Y). The amino acids at positions 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 16 were varied to permit any amino acid except cysteine (Cys).

The LN20 library was constructed to display multiple linear peptides on the surface of a phage. Each phage, however, displays multiple copies of the same sequence. Therefore, a single phage will display, for example, five copies of a particular sequence, a different phage will display, for example, five copies of a different sequence, etc. The linear peptides are provided in a 20-amino acid template. The amino acids at each position in the template were varied to permit any amino acid except cysteine (Cys).

The binding polypeptides provided herein can include additions or truncations in the – and/or C- termini. Such modified binding polypeptides are expected to bind cMet. For example, a -GGGK linker (SEQ ID NO:513) can be present at the N-terminus of the binding polypeptides provided herein. Other linkers, such as –GSGK(SEQ ID NO:651), or –GSGSK(SEQ ID NO:652) could be used. Binding polypeptides comprising the loop portion of the templates and sequences provided herein are expected to bind cMet and also are encompassed by the present invention. The loop portion of the templates and sequences includes the sequences between and including the two cysteine residues that are expected to form a disulfide bond, thereby generating a peptide loop structure. Furthermore, the binding polypeptides of the present invention can include additional amino acid residues at the – and/or C-termini.

The phage display libraries were created by making a designed series of mutations or variations within a coding sequence for the polypeptide template, each mutant sequence encoding a peptide analog corresponding in overall structure to the template except having one or more amino acid variations in the sequence of the template. The novel variegated (mutated) DNA provides sequence diversity, and each transformant phage displays one variant of the initial template amino acid sequence encoded by the DNA, leading to a phage population (library) displaying a vast number of different but structurally related amino acid sequences. The amino acid variations

are expected to alter the binding properties of the binding peptide or domain without significantly altering its structure, at least for most substitutions. It is preferred that the amino acid positions that are selected for variation (variable amino acid positions) will be surface amino acid positions, that is, positions in the amino acid sequence of the domains that, when the domain is in its most stable conformation, appear on the outer surface of the domain (*i.e.*, the surface exposed to solution). Most preferably the amino acid positions to be varied will be adjacent or close together, so as to maximize the effect of substitutions.

As indicated previously, the techniques discussed in Kay *et al.*, *Phage Display of Peptides and Proteins: A Laboratory Manual* (Academic Press, Inc., San Diego, 1996) and US 5,223,409 are particularly useful in preparing a library of potential binders corresponding to the selected parental template. Libraries as discussed above were prepared according to such techniques, and they were screened for cMet binding polypeptides against an immobilized target, as explained in the examples to follow.

In a typical screen, a phage library is contacted with and allowed to bind the target, or a particular subcomponent thereof. To facilitate separation of binders and non-binders, it is convenient to immobilize the target on a solid support. Phage bearing a target-binding moiety form a complex with the target on the solid support whereas non-binding phage remain in solution and can be washed away with excess buffer. Bound phage are then liberated from the target by changing the buffer to an extreme pH (pH 2 or pH 10), changing the ionic strength of the buffer, adding denaturants, or other known means. To isolate the binding phage exhibiting the polypeptides of the present invention, a protein elution is performed, *i.e.*, some phage are eluted from the target using HGF in solution (competitive elution). Additionally, for example, very high affinity binding phage that could not be competed off during the overnight HGF incubation were captured by using the phage still bound to substrate for infection of *E. coli* cells.

The recovered phage can then be amplified through infection of bacterial cells and the screening process can be repeated with the new pool that is now depleted in non-binders and enriched for binders. The recovery of even a few binding phage is sufficient to carry the process to completion. After a few rounds of selection, the gene

sequences encoding the binding moieties derived from selected phage clones in the binding pool are determined by conventional methods, described below, revealing the peptide sequence that imparts binding affinity of the phage to the target. When the selection process works, the sequence diversity of the population falls with each round of selection until desirable binders remain. The sequences converge on a small number of related binders, typically 10-50 out of about 10^9 to 10^{10} original candidates from each library. An increase in the number of phage recovered at each round of selection, and of course, the recovery of closely related sequences are good indications that convergence of the library has occurred in a screen. After a set of binding polypeptides is identified, the sequence information can be used to design other secondary phage libraries, biased for members having additional desired properties.

Formation of the disulfide binding loop is advantageous because it leads to increased affinity and specificity for such peptides. However, in serum, the disulfide bond can be opened by free cysteines or other thiol-containing molecules. Thus, it could be useful to modify the cysteine residues to replace the disulfide cross-link with another less reactive linkage. The $-CH_2-S-S-CH_2-$ cross-link has a preferred geometry in which the dihedral bond between sulfurs is close to 90 degrees, but the exact geometry is determined by the context of other side groups and the binding state of the molecule. Preferred modifications of the closing cross-link of the binding loop will preserve the overall bond lengths and angles as much as possible. Suitable such alternative cross-links include thioether linkages such as $-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-S-CH_2-$, $-CH_2-CH_2-S-CH_2-CH_2-$; lactam or amide linkages such as $-CH_2-NH-CO-CH_2-$ and $-CH_2-CO-NH-CH_2-$; ether linkages such as $-CH_2-CH_2-O-CH_2-CH_2-$; alkylene bridges such as $-(CH_2)_n-$ (where n = 4, 5, or 6); the linkage $-CH_2-NH-CO-NH-CH_2-$, and similar groups known in the art.

Although polypeptides containing a stable disulfide-linked binding loop are most preferred, linear polypeptides derived from the foregoing sequences can be readily prepared, *e.g.*, by substitution of one or both cysteine residues, which may retain at least some of the cMet binding activity of the original polypeptide containing the disulfide linkage. In making such substitutions for Cys, the amino acids Gly, Ser, and Ala are preferred, and it also is preferred to substitute both Cys residues, so as not to leave a

single Cys that could cause the polypeptide to dimerize or react with other free thiol groups in a solution. All such linearized derivatives that retain cMet binding properties are within the scope of this invention.

Direct synthesis of the polypeptides of the invention can be accomplished using conventional techniques, including solid-phase peptide synthesis, solution-phase synthesis, etc. Solid-phase synthesis is preferred (see, for example, Stewart *et al.*, *Solid-Phase Peptide Synthesis* (W. H. Freeman Co., San Francisco, 1989); Merrifield, J., 1963, *Am. Chem. Soc.*, 85:2149-2154; Bodanszky and Bodanszky, *The Practice of Peptide Synthesis* (Springer-Verlag, New York, 1984)), incorporated herein by reference.

Polypeptides according to the invention can also be prepared commercially by companies providing peptide synthesis as a service (*e.g.*, BACHEM Bioscience, Inc., King of Prussia, PA; Quality Controlled Biochemicals, Inc., Hopkinton, MA).

Automated peptide synthesis machines, such as manufactured by Perkin-Elmer Applied Biosystems, also are available.

The polypeptide compound is preferably purified after it has been isolated or synthesized by either chemical or recombinant techniques. For purification purposes, there are many standard methods that may be employed, including reversed-phase high pressure liquid chromatography (RP-HPLC) using an alkylated silica column such as C_4-, C_8- or C_{18}-silica. A gradient mobile phase of increasing organic content is generally used to achieve purification, for example, acetonitrile in an aqueous buffer, usually containing a small amount of trifluoroacetic acid. Ion-exchange chromatography can also be used to separate peptides based on their charge. The degree of purity of the polypeptide can be determined by various methods, including identification of a major large peak on HPLC. A polypeptide that produces a single peak that is at least 95% of the input material on an HPLC column is preferred. Even more preferable is a polypeptide that produces a single peak that is at least 97%, at least 98%, at least 99% or even 99.5% or more of the input material on an HPLC column.

To ensure that the peptide obtained using any of the techniques described above is the desired peptide for use in compositions of the present invention, analysis of the peptide composition can be carried out. Such composition analysis can be conducted

using high resolution mass spectrometry to determine the molecular weight of the peptide. Alternatively, the amino acid content of the peptide can be confirmed by hydrolyzing the peptide in aqueous acid, and separating, identifying and quantifying the components of the mixture using HPLC, or an amino acid analyzer. Protein sequenators, which sequentially degrade the peptide and identify the amino acids in order, can also be used to determine the sequence of the peptide.

cMet binding polypeptides according to the present invention also can be produced using recombinant DNA techniques, utilizing nucleic acids (polynucleotides) encoding the polypeptides according to this invention and then expressing them recombinantly, *i.e.*, by manipulating host cells by introduction of exogenous nucleic acid molecules in known ways to cause such host cells to produce the desired cMet binding polypeptides. Such procedures are within the capability of those skilled in the art (see, for example, Davis *et al.*, *Basic Methods in Molecular Biology* (1986)), incorporated by reference. Recombinant production of short peptides, such as those described herein, might not be practical in comparison to direct synthesis, however recombinant means of production can be very advantageous where a cMet binding moiety of this invention is incorporated in a hybrid polypeptide or fusion protein.

In the practice of the present invention, a determination of the affinity of the cMet binding moiety for cMet relative to another protein or target is a useful measure, and is referred to as specificity for cMet. Standard assays for quantitating binding and determining affinity include equilibrium dialysis, equilibrium binding, gel filtration, or the monitoring of numerous spectroscopic changes (such as a change in fluorescence polarization) that result from the interaction of the binding moiety and its target. These techniques measure the concentration of bound and free ligand as a function of ligand (or protein) concentration. The concentration of bound polypeptide ([Bound]) is related to the concentration of free polypeptide ([Free]) and the concentration of binding sites for the polypeptide, *i.e.*, on cMet, (N), as described in the following equation:

$$[\text{Bound}] = N \times [\text{Free}]/((1/K_a)+[\text{Free}]).$$

A solution of the data to this equation yields the association constant, K_a, a quantitative measure of the binding affinity. The association constant, K_a is the reciprocal of the dissociation constant, K_D. The K_D is more frequently reported in measurements of affinity. Preferred cMet binding polypeptides have a K_D for cMet in the range of, for example, less than 1 nanomolar (nM), 1 nM to 100 micromolar (μM), which includes K_D values of less than 10 nM, less than 20 nM, less than 40 nM, less than 60 nM, less than 80 nM, less than 1 μM, less than 5 μM, less than 10 μM, less than 20 μM, less than 40 μM, less than 60 μM, and less than 80 μM.

Where cMet binding moieties are employed as imaging agents, other aspects of binding specificity become important; imaging agents operate in a dynamic system in that binding of the imaging agent to the target (cMet, *e.g.*, on activated cells) might not be in a stable equilibrium state throughout the imaging procedure. For example, when the imaging agent is initially injected, the concentration of imaging agent and of agent-target complex rapidly increases. Shortly after injection, however, the circulating (free) imaging agent starts to clear through the kidneys or liver, and the plasma concentration of imaging agent begins to drop. This drop in the concentration of free imaging agent in the plasma eventually causes the agent-target complex to dissociate. The usefulness of an imaging agent depends on the difference in rate of agent-target dissociation relative to the clearing rate of the agent. Ideally, the dissociation rate will be slow compared to the clearing rate, resulting in a long imaging time during which there is a high concentration of agent-target complex and a low concentration of free imaging agent (background signal) in the plasma.

Quantitative measurement of dissociation rates can be performed using several methods known in the art, such as fiber optic fluorimetry (see, for example, Anderson and Miller, 1988, *Clin. Chem.*, 34:1417-21), surface plasmon resonance (see, for example, Malmborg *et al.*, 1996, *J. Immunol. Methods*, 198:51-7; and Schuck, 1997, *Curr. Op. Biotechnol.*, 8:498-502), resonant mirror, and grating coupled planar waveguiding (see, for example, Hutchinson, 1995, *Molec. Biotechnol.*, 3:47-54). Automated biosensors are commercially available for measuring binding kinetics: BIAcore surface plasmon resonance sensor (Biacore AB, Uppsala SE), IAsys resonant

mirror sensor (Fisons Applied Sensor Technology, Cambridge GB), BIOS-1 grated coupled planar waveguiding sensor (Artificial Sensor Instruments, Zurich CH).

Methods of Screening Polypeptides Identified by Phage Display For Their Ability To Bind To Cells Expressing The Target

In another aspect of the invention, methods of screening binding polypeptides identified by phage display for their ability to bind to cells expressing the target (and not to cells that do not express the target) are provided. These methods address a significant problem associated with screening peptides identified by phage display: frequently the peptides so identified do not have sufficient affinity for the target to be screened against target-expressing cells in conventional assays. However, ascertaining that a particular phage-identified peptide binds to cells that express the target (and does not bind to cells that do not) is a critical piece of information in identifying binding peptides that are potential *in vivo* targeting moieties, whether they are used as monomers or as part of a multimeric construct. The method takes advantage of the increase in affinity and avidity associated with multivalent binding and permit screening of polypeptides with low affinities against target-expressing cells.

The method generally consists of preparation and screening of multimeric constructs including one or more binding polypeptides. For example, polypeptides identified by phage display as binding to a target are biotinylated and complexed with avidin, streptavidin or neutravidin to form tetrameric constructs. These tetrameric constructs are then incubated with cells that express the desired target and cells that do not, and binding of the tetrameric construct is detected. Binding can be detected using any method of detection known in the art. For example, to detect binding the avidin, streptavidin, or neutravidin may be conjugated to a detectable marker (*e.g.*, a radioactive label, a fluorescent label, or an enzymatic label which undergoes a color change, such as HRP (horse radish peroxidase), TMB (tetramethyl benzidine) or alkaline phosphatase).

The biotinylated peptides are preferably complexed with neutravidin-HRP. Neutravidin exhibits lower non-specific binding to molecules than the other alternatives due to the absence of lectin binding carbohydrate moieties and cell adhesion receptor-

binding RYD domain in neutravidin (Hiller, Y. *et al.*, 1987. *Biochem. J.*, 248:167-171; Alon, R. *et al.*, 1990. *Biochem. Biophys. Res. Commun.*, 170:1236-41).

The tetrameric constructs can be screened against cells that naturally express the target or cells that have been engineered via recombinant DNA technologies to express the target (*e.g.*, transfectants, transformants, etc.). If cells that have been transfected to express the target are used, mock transfected cells (*i.e.*, cells transfected without the genetic material encoding the target) can be used as a control.

The tetrameric complexes can optionally be screened in the presence of serum. Thus, the assay also can be used to rapidly evaluate the effect of serum on the binding of peptides to the target.

The methods disclosed herein are particularly useful in preparing and evaluating combinations of distinct binding polypeptides for use in dimeric or multimeric targeting constructs that contain two or more binding polypeptides. Use of biotin/avidin complexes allows for relatively easy preparation of tetrameric constructs containing one to four different binding peptides. Furthermore, it has now been found that affinity and avidity of a targeting construct can be increased by inclusion of two or more targeting moieties that bind to different epitopes on the same target. The screening methods described herein are useful in identifying combinations of binding polypeptides that could have increased affinity when included in such multimeric constructs.

In a preferred embodiment, the screening methods described herein can be used to screen cMet binding polypeptides identified by phage display, such as those described herein. These methods can be used to assess the specific binding of cMet binding polypeptides to cells that express cMet or have been engineered to express cMet. Tetrameric complexes of biotinylated cMet binding polypeptides of the invention and, for example, neutravidin-HRP can be prepared and screened against cells transfected to express cMet as well as mock transfected cells, which do not express cMet.

The assay can be used to identify cMet binding polypeptides that bind specifically to cMet-expressing cells (and do not bind to cells that do not express cMet) even when the monodentate K_D of the polypeptide is on the order of 200 nM-300 nM. The assay can be used to screen homotetrameric constructs containing four copies of a

single cMet binding polypeptide of the invention as well as heterotetrameric (constructs containing two or more different cMet binding polypeptides). The methods described herein are particularly useful for assessing combinations of cMet binding polypeptides for use in multimeric constructs, particularly constructs containing two or more cMet binding polypeptides that bind to different epitopes of cMet.

The assay also can be used to assess the effect of serum on the cMet binding polypeptides.

Modification or Optimization of cMet Binding Polypeptides

As discussed, modification or optimization of cMet binding polypeptides is within the scope of the invention and the modified or optimized polypeptides are included within the definition of "cMet binding polypeptides". Specifically, a polypeptide sequence identified by phage display can be modified to optimize its potency, pharmacokinetic behavior, stability and/or other biological, physical and chemical properties.

Substitution of Amino Acid Residues

For example, one can make the following isosteric and/or conservative amino acid changes in the parent polypeptide sequence with the expectation that the resulting polypeptides would have a similar or improved profile of the properties described above:

Substitution of alkyl-substituted hydrophobic amino acids: including alanine, leucine, isoleucine, valine, norleucine, S-2-aminobutyric acid, S-cyclohexylalanine or other simple alpha-amino acids substituted by an aliphatic side chain from C1-10 carbons including branched, cyclic and straight chain alkyl, alkenyl or alkynyl substitutions.

Substitution of aromatic-substituted hydrophobic amino acids: including phenylalanine, tryptophan, tyrosine, biphenylalanine, 1-naphthylalanine, 2-naphthylalanine, 2-benzothienylalanine, 3-benzothienylalanine, histidine, amino, alkylamino, dialkylamino, aza, halogenated (fluoro, chloro, bromo, or iodo) or alkoxy

(from C_1-C_4)-substituted forms of the previous listed aromatic amino acids, illustrative examples of which are: 2-,3- or 4-aminophenylalanine, 2-,3- or 4-chlorophenylalanine, 2-,3- or 4-methylphenylalanine, 2-,3- or 4-methoxyphenylalanine, 5-amino-, 5-chloro-, 5-methyl- or 5-methoxytryptophan, 2'-, 3'-, or 4'-amino-, 2'-, 3'-, or 4'-chloro-, 2,3,or 4-biphenylalanine, 2',-3',-or 4'- methyl-2,3 or 4-biphenylalanine, and 2- or 3-pyridylalanine.

Substitution of amino acids containing basic functions: including arginine, lysine, histidine, ornithine, 2,3-diaminopropionic acid, homoarginine, alkyl, alkenyl, or aryl-substituted (from C_1-C_{10} branched, linear, or cyclic) derivatives of the previous amino acids, whether the substituent is on the heteroatoms (such as the alpha nitrogen, or the distal nitrogen or nitrogens, or on the alpha carbon, in the pro-R position for example. Compounds that serve as illustrative examples include: N-epsilon-isopropyl-lysine, 3-(4-tetrahydropyridyl)-glycine, 3-(4-tetrahydropyridyl)-alanine, N,N-gamma, gamma'-diethyl-homoarginine. Included also are compounds such as alpha methyl arginine, alpha methyl 2,3-diaminopropionic acid, alpha methyl histidine, alpha methyl ornithine where alkyl group occupies the pro-R position of the alpha carbon. Also included are the amides formed from alkyl, aromatic, heteroaromatic (where the heteroaromatic group has one or more nitrogens, oxygens or sulfur atoms singly or in combination) carboxylic acids or any of the many well-known activated derivatives such as acid chlorides, active esters, active azolides and related derivatives) and lysine, ornithine, or 2,3-diaminopropionic acid.

Substitution of acidic amino acids: including aspartic acid, glutamic acid, homoglutamic acid, tyrosine, alkyl, aryl, arylalkyl, and heteroaryl sulfonamides of 2,4-diaminopriopionic acid, ornithine or lysine and tetrazole-substituted alkyl amino acids.

Substitution of side chain amide residues: including asparagine, glutamine, and alkyl or aromatic substituted derivatives of asparagine or glutamine.

Substitution of hydroxyl containing amino acids: including serine, threonine, homoserine, 2,3-diaminopropionic acid, and alkyl or aromatic substituted derivatives of serine or threonine. It is also understood that the amino acids within each of the categories listed above can be substituted for another of the same group.

Substitution of Amide Bonds

Another type of modification within the scope of the invention is to substitute the amide bonds within the backbone of the polypeptide. For example, to reduce or eliminate undesired proteolysis, or other degradation pathways that diminish serum stability, resulting in reduced or abolished bioactivity, or to restrict or increase conformational flexibility, one can substitute amide bonds within the backbone of the peptides with functionality that mimics the existing conformation or alters the conformation in the manner desired. Such modifications can produce increased binding affinity or improved pharmacokinetic behavior. It is understood that those knowledgeable in the art of peptide synthesis can make the following amide bond changes for any amide bond connecting two amino acids with the expectation that the resulting peptides could have the same or improved activity: insertion of alpha-N-methylamides or peptide amide backbone thioamides, removal of the carbonyl to produce the cognate secondary amines, replacement of one amino acid with an aza-amino acid to produce semicarbazone derivatives, and use of E-olefins and substituted E-olefins as amide bond surrogates.

Introduction of D-Amino Acids

Another approach within the scope of the invention is the introduction of D-alanine, or another D-amino acid, distal or proximal to the labile peptide bond. In this case it is also understood to those skilled in the art that such D-amino acid substitutions can, and at times, must be made, with D-amino acids whose side chains are not conservative replacements for those of the L-amino acid being replaced. This is because of the difference in chirality and hence side-chain orientation, which could result in the accessing of a previously unexplored region of the binding site of the target

that has moieties of different charge, hydrophobicity, steric requirements etc. than that serviced by the side chain of the replaced L-amino acid.

Modifications To Improve Pharmacokinetic or Pharmacodynamic Properties

It also is understood that use of one or more cMet binding polypeptides in a particular application could be benefitted by modifications of the peptide or formulations of the peptide to improve pharmacokinetic and pharmacodynamic behavior. It is expected that the properties of the peptide can be changed by attachment of moieties anticipated to bring about the desired physical or chemical properties. Such moieties can be appended to the peptide using acids or amines, via amide bonds or urea bonds, respectively, to the – or C-terminus of the peptide, or to the pendant amino group of a suitably located lysine or lysine derivative, 2, 3-diaminopropionic acid, ornithine, or other amino acid in the peptide that possesses a pendant amine group or a pendant alkoxyamine or hydrazine group. Conversely acidic amino acid side-chains such as those of Asp or Glu can be selectively unmasked and amidated with amines bearing the desired modifying functionality, or they can be modified in this manner before incorporation into the peptide chain. The moieties introduced can be groups that are hydrophilic, basic, or nonpolar alkyl or aromatic groups depending on the peptide of interest and the extant requirements for modification of its properties.

Glycosylation of Amino Acid Residues

Yet another modification within the scope of the invention is glycosylation of one or more amino acid residues (*e.g.*, serine or threonine residues) in the cMet binding polypeptide. Glycosylation, which can be carried out using standard conditions, can be used to enhance solubility, alter pharmacokinetics and pharmacodynamics or to enhance binding via a specific or non-specific interaction involving the glycosidic moiety.

Formation of Salts

It also is within the scope of the invention to form different salts that could increase or decrease the water solubility or the ease of formulation of these peptides.

These may include, but are not restricted to, N-methylglucamine (meglumine), acetate, oxalates, ascorbates, etc.

Structural Modifications which Retain Structural Features

Yet another modification within the scope of the invention is truncation of cyclic polypeptides. The cyclic nature of many polypeptides of the invention limits the conformational space available to the peptide sequence, particularly within the cycle. Therefore truncation of the peptide by one or more residues distal or even proximal to the cycle, at either the N-terminal or C-terminal region could provide truncated peptides with similar or improved biological activity. A unique sequence of amino acids, even as small as three amino acids, which is responsible for the binding activity, can be identified, as noted for RGD peptides (Plow, E. *et al.*, 1987. *Blood*, 70:110-5; Oldberg, A. *et al.*, 1988. *J. Biol. Chem.*, 263:19433-19436; Taub, R. *et al.*, 1989. *J. Biol. Chem.*, 264:259-65; Andrieux, A. *et al.*, 1989. *J. Biol. Chem.*, 264:9258-65; and U.S. Patent Nos. 5,773,412 and 5,759,996, each of which is incorporated herein by reference).

It also has been shown in the literature that large peptide cycles can be substantially shortened, eliminating extraneous amino acids, but substantially including the critical binding residues. See, U.S. Patent No. 5,556,939, incorporated by reference herein.

The shortened cyclic peptides can be formed using disulfide bonds or amide bonds of suitably located carboxylic acid groups and amino groups.

Furthermore, D-amino acids can be added to the peptide sequence to stabilize turn features (especially in the case of glycine). In another approach alpha, beta, gamma or delta dipeptide or turn mimics (such as α, β, γ, or δ turn mimics), some of which are shown in FIGS. 1A-1C, can be employed to mimic structural motifs and turn features in a peptide and simultaneously provide stability from proteolysis and enhance other properties such as, for example, conformational stability and solubility (structure 1A: Hart et al., J. Org. Chem., 64, 2998-2999(1999); structure 1B: Hanessian et al., "Synthesis of a Versatile Peptidomimetic Scaffold" in Methods in Molecular Medicine, Vol. 23: Peptidomimetics Protocols, W. Kazmierski, Ed. (Humana Press Inc., Totowa, N.J., 1999), Chapter 10, pp. 161-174; structure 1C: WO 01/16135.

Substitution of Disulfide Mimetics

Also within the scope of the invention is the substitution of disulfide mimetics for disulfide bonds within the cMet binding peptides of the invention.

Where disulfide-containing peptides are employed in generating ^{99m}Tc-based radiopharmaceuticals, or other useful radiopharmaceuticals based on other isotopes, a significant problem is the presence of the disulfide bond. For example, the integrity of the disulfide bond is difficult to maintain during procedures designed to incorporate ^{99m}Tc via routes that are reliant upon the reduction of pertechnetate ion and subsequent incorporation of the reduced Tc species into substances bearing Tc-specific chelating groups. This is because the disulfide bond is rather easily reduced by the reducing agents commonly used in kits devised for one-step preparation of radiopharmaceuticals. Therefore, the ease with which the disulfide bond can be reduced during Tc chelation may require substitution with mimetics of the disulfide bonds. Accordingly, another modification within the scope of the invention is to substitute the disulfide moiety with mimetics utilizing the methods disclosed herein or known to those skilled in the art, while retaining the activity and other desired properties of the cMet-binding polypeptides of the invention.

1.) Oxime linker

The oxime moiety has been employed as a linker by investigators in a number of contexts (Wahl, F. and Mutter, M., 1996. *Tetrahedron Lett.*, 37:6861-6864). As shown in FIG. 2, the amino acids 4, containing an aminoalcohol function, and 5 containing an alkoxyamino function, can be incorporated into the peptide chain, not necessarily at the end of the peptide chain. After formation of the peptide the side-chain protecting groups can be removed. The aldehyde group is then unmasked and an oxime linkage is formed.

2.) Lanthionine Linker

Lanthionines are cyclic sulfides, wherein the disulfide linkage (S-S) is replaced by a carbon-sulfur (C-S) linkage. Thus, the lability to reduction is far lower. Lanthionines can be prepared by a number of methods including those discussed below.

1) Preparation of Lanthionines using Bromoacetylated Peptides

Lanthionines can be readily prepared using known methods (Robey, F. and Fields, R., 1989. *Anal. Biochem.*, 177:373-377; Inman, J. *et al.*, 1991. *Bioconjug. Chem.*, 2:458-463; Ploinsky, A. *et al.*, 1992. *J. Med. Chem.*, 35:4185-4194; Mayer et al., "Peptides, Frontiers of Peptide Science", in *Proceedings of the 15th American Peptide Symposium*, Tam and Kaumaya (Eds.), June 14-19, 1995, Nashville, Tenn. (Klumer Academic Pub., Boston), pp. 291-292; Wakao *et al.*, Jpn. Kokai Tokyo Koho, JP 07300452 A2 (1995)). Preparation of peptides using Boc automated peptide synthesis followed by coupling the peptide terminus with bromoacetic acid gives bromoacetylated peptides in good yield. Cleavage and deprotection of the peptides can be accomplished using HF/anisole. If the peptide contains a cysteine group its reactivity can be controlled with low pH. If the pH of the medium is raised to 6-7 then either polymerization or cyclization of the peptide takes place. Polymerization is favored at high (100 mg/mL) concentration whereas cyclization is favored at lower concentrations (1 mg/mL), *e.g.*, 6 cyclizes to 7 (referred to herein as "scheme 1" as shown in FIG. 3). Inman *et al.* demonstrated the use of Na-(Boc)-Ne-[N-(bromoacetyl)-β-alanyl]-L-lysine as a carrier of the bromoacetyl group that could be employed in Boc peptide synthesis thus allowing placement of a bromoacetyl bearing moiety anywhere in a sequence. In preliminary experiments they found that peptides with 4-6 amino acids separating the bromoacetyl-lysine derivative from a cysteine tend to cyclize, indicating the potential utility of this strategy.

2) Preparation of Lanthionines via Cysteine Thiol Addition to Acrylamides

Several variants of this strategy can be implemented. Resin-bound serine can be employed to prepare the lanthionine ring on resin either using a bromination-dehydrobromination-thiol addition sequence or by dehydration with disuccinimidyl

carbonate followed by thiol addition. Conjugate addition of thiols to acrylamides has also been amply demonstrated and a reference to the addition of 2-mercaptoethanol to acrylamide is provided (Wakao *et al.*, Jpn. Kokai Tokyo Koho, JP 07300452 A2, 1995).

3) Diaryl Ether or Diarylamine Linkage From Intramolecular Cyclization of Aryl Boronic Acids and Tyrosine

The reaction of arylboronic acids with phenols, amines and heterocyclic amines in the presence of cupric acetate, in air, at ambient temperature, in dichloromethane using either pyridine or triethylamine as a base to provide unsymmetrical diaryl ethers and the related amines in good yields (as high as 98%) has been reported (Evans, D. *et al.*, 1998. *Tetrahedron Lett.*, 39:2937-2940; Chan, D. *et al.*, 1998. *Tetrahedron Lett.*, 39:2933-2936; Lam, P. *et al.*, 1998. *Tetrahedron Lett.*, 39:2941-2944). In the case of N-protected tyrosine derivatives as the phenol component the yields were also as high as 98%. This demonstrates that amino acid amides (peptides) are expected to be stable to the transformation and that yields are high. Precedent for an intramolecular reaction exists in view of the facile intramolecular cyclizations of peptides to lactams, intramolecular biaryl ether formation based on the SNAr reaction and the generality of intramolecular cyclization reactions under high dilution conditions or on resin, wherein the pseudo-dilution effect mimics high dilution conditions.

4) Formation of Cyclic Peptides with a Thiazolidine Linkage via Intramolecular Reaction of Peptide Aldehydes with Cysteine Moieties

Another approach that may be employed involves intramolecular cyclization of suitably located vicinal amino mercaptan functions (usually derived from placement of a cysteine at a terminus of the linear sequence or tethered to the sequence via a side-chain nitrogen of a lysine, for example) and aldehyde functions to provide thiazolidines that result in the formation of a bicyclic peptide, one ring of which is that formed by the residues in the main chain, and the second ring being the thiazolidine ring. Scheme 2 (FIG. 4) provides an example. The required aldehyde function can be generated by sodium metaperiodate cleavage of a suitably located vicinal aminoalcohol function,

which can be present as an unprotected serine tethered to the chain by appendage to a side chain amino group of a lysine moiety. In some cases the required aldehyde function is generated by unmasking of a protected aldehyde derivative at the C-terminus or the N-terminus of the chain (Botti, P. *et al.*, 1996. *J. Am. Chem. Soc.*, 118:10018-10034).

5) Lactams Based on Intramolecular Cyclization of Pendant Amino Groups with Carboxyl Groups on Resin.

Macrocyclic peptides can be prepared by lactam formation by either head-to-tail or by pendant group cyclization. The basic strategy is to prepare a fully protected peptide wherein it is possible to remove selectively an amine protecting group and a carboxy protecting group. Orthogonal protecting schemes have been developed. Of those that have been developed the allyl, trityl and Dde methods have been employed most (Mellor *et al.*, "Synthesis of Modified Peptides", in *Fmoc Solid Phase Synthesis: A Practical Approach*, White and Chan (eds) (Oxford University Press, New York, 2000), Ch. 6, pp. 169-178). The Dde approach is of interest because it utilizes similar protecting groups for both the carboxylic acid function (Dmab ester) and the amino group (Dde group). Both are removed with 2-10% hydrazine in DMF at ambient temperature. Alternately the Dde can be used for the amino group and the allyl group can be used for the carboxyl.

A lactam function, available by intramolecular coupling via standard peptide coupling reagents (such as HATU, PyBOP etc) can act as a surrogate for the disulfide bond. The Dde/Dmab approach is shown in Scheme 3 (FIG. 5).

Thus, a linear sequence containing, for example, the Dde-protected lysine and Dmab ester can be prepared on a Tentagel-based Rink amide resin at low load (~0.1-0.2 mmol/g). Deprotection of both functions with hydrazine is then followed by on-resin cyclization to give the desired products. Subsequently cleavage from resin and purification may also be carried out. For functionalization of the N-terminus of the peptide it is understood that diamino acids such as trans-4-(iv-Dde)methylaminocyclohexane carboxylic acid or 4-(iv-Dde)methylamino benzoic acid

would be required. An alternative scenario is to employ the safety catch method to intramolecular lactam formation during cleavage from the resin.

6) Cyclic Peptides Based on Olefin Metathesis

The Grubbs reaction (Scheme 4, FIG. 6) involves the metathesis/cyclization of olefin bonds (Schuster *et al.*, 1997. *Angew. Chem. Int. Edn Engl.*, 36:2036-2056; Miller *et al.*, 1996. *J. Am. Chem. Soc.*, 118:9606-9614). It is readily seen that if the starting material is a diolefin 16 that the resulting product will be cyclic compound 17. The reaction has been applied to creation of cycles from olefin-functionalized peptides (Pernerstorfer *et al.*, 1997. *Chem. Commun.*, 20:1949-50; Clark *et al.*, 1999. *Chem.Eur. J.*, 5:782-792; Blackwell *et al.*, 1998 *Angew. Chem. Int. Ed.*, 37:3281-3284; Ripka, A. *et al.*, 1998. *Bioorg. Med. Chem. Lett.*, 8:357-360; Miller *et al.*, 1996. *J. Am. Chem. Soc.*, 118:9606-9614; Clark *et al.*, 1995. *J. Am. Chem. Soc.*, 117:12364-12365; Miller *et al.*, 1995. *J. Am. Chem. Soc.*, 117:5855-5856). One can prepare either C-allylated amino acids or possibly N-allylated amino acids and employ them in this reaction in order to prepare carba-bridged cyclic peptides as surrogates for disulfide bond containing peptides.

One also can prepare novel compounds with olefinic groups. Functionalization of the tyrosine hydroxyl with an olefin-containing tether is one option. The lysine ε-amino group is another option with appendage of the olefin-containing unit as part of an acylating moiety, for example. If instead the lysine side chain amino group is alkylated with an olefin containing tether, it can still function as a point of attachment for a reporter as well. The use of 5-pentenoic acid as an acylating agent for the lysine, ornithine, or diaminopropionic side chain amino groups is another possibility. The length of the olefin-containing tether can also be varied in order to explore structure activity relationships.

Manipulation of Peptide Sequences

Other modifications within the scope of the invention include manipulations of peptide sequences, which can be expected to yield peptides with similar or improved biological properties. These include amino acid translocations (swapping amino acids

in the sequence), use of retro-inverso peptides in place of the original sequence or a modified original sequence, peptoids and retro-inverso peptoid sequences. Structures wherein specific residues are peptoid instead of peptidic, which result in hybrid molecules, neither completely peptidic nor completely peptoid, are anticipated as well.

Linkers

Additionally, modifications within the invention include introduction of linkers or spacers between the targeting sequence of the binding moiety or binding polypeptide and the detectable label or therapeutic agent. For example, use of such linkers/spacers can improve the relevant properties of the binding peptides (*e.g.*, increase serum stability, etc.). These linkers can include, but are not restricted to, substituted or unsubstituted alkyl chains, polyethylene glycol derivatives, amino acid spacers, sugars, or aliphatic or aromatic spacers common in the art.

For example, suitable linkers include homobifunctional and heterobifunctional cross-linking molecules. The homobifunctional molecules have at least two reactive functional groups, which are the same. The reactive functional groups on a homobifunctional molecule include, for example, aldehyde groups and active ester groups. Homobifunctional molecules having aldehyde groups include, for example, glutaraldehyde and subaraldehyde.

Homobifunctional linker molecules having at least two active ester units include esters of dicarboxylic acids and N-hydroxysuccinimide. Some examples of such N-succinimidyl esters include disuccinimidyl suberate and dithio-bis-(succinimidyl propionate), and their soluble bis-sulfonic acid and bis-sulfonate salts such as their sodium and potassium salts.

Heterobifunctional linker molecules have at least two different reactive groups. Some examples of heterobifunctional reagents containing reactive disulfide bonds include N-succinimidyl 3-(2-pyridyl-dithio)propionate (Carlsson *et al.*, 1978. *Biochem. J.*, 173:723-737), sodium S-4-succinimidyloxycarbonyl-alpha-methylbenzylthiosulfate, and 4-succinimidyloxycarbonyl-alpha-methyl-(2-pyridyldithio)toluene. N-succinimidyl 3-(2-pyridyldithio)propionate is preferred. Some examples of heterobifunctional reagents comprising reactive groups having a double bond that reacts with a thiol group

include succinimidyl 4-(N-maleimidomethyl)cyclohexahe-1-carboxylate and succinimidyl m-maleimidobenzoate. Other heterobifunctional molecules include succinimidyl 3-(maleimido)propionate, sulfosuccinimidyl 4-(p-maleimido-phenyl)butyrate, sulfosuccinimidyl 4-(N-maleimidomethyl-cyclohexane)-1-carboxylate, maleimidobenzoyl-5N-hydroxy-succinimide ester.

Furthermore, linkers that are combinations of the molecules and/or moieties described above, can also be employed to confer special advantage to the properties of the peptide. Lipid molecules with linkers may be attached to allow formulation of ultrasound bubbles, liposomes or other aggregation based constructs. Such constructs could be employed as agents for targeting and delivery of a diagnostic reporter, a therapeutic agent (*e.g.*, a chemical "warhead" for therapy), or a combination of these.

Multimeric Constructs of cMet Binding Polypeptides

Constructs employing dimers, multimers or polymers of one or more cMet binding polypeptides of the invention are also contemplated. Indeed, there is ample literature evidence that the binding of low potency peptides or small molecules can be substantially increased by the formation of dimers and multimers. Thus, dimeric and multimeric constructs (both homogeneous and heterogeneous) are within the scope of the instant invention. The polypeptide sequences in the dimeric constructs can be attached at their N- or C- terminus or the N-epsilon nitrogen of a suitably placed lysine moiety (or another function bearing a selectively derivatizable group such as a pendant oxyamino or other nucleophilic group), or can be joined together via one or more linkers (*e.g.*, those discussed herein) employing the appropriate attachment chemistry. This coupling chemistry can include amide, urea, thiourea, oxime, or aminoacetylamide (from chloro- or bromoacetamide derivatives, but is not so limited). For example, methods to prepare dimeric or multimeric constructs of cMet binding polypeptides of the invention include at least those discussed below.

Method A

Fully protected cMet-binding peptides can be built up on Ellman-type safety catch resin using automated or manual Fmoc peptide synthesis protocols (Backes *et al.*,

1996. *J. Am. Chem. Soc.*, 118:3055-56). Separately, using standard methods known in the art of peptide synthesis, a di-lysine derivative can be constructed on 2-chlorotrityl resin (Fields *et al.*, "Principles and Practice of Solid Phase Synthesis" in *Synthetic Peptides, A Users Guide*, Grant, Ed. (W.H. Freeman Co., New York, 1992), Ch. 3, pp. 77-183; Barlos *et al.*, "Convergent Peptide Synthesis" in *Fmoc Solid Phase Peptide Synthesis*, Chan, W.C. and White, P.D., Eds. (Oxford University Press, New York, 2000), Ch. 9, pp. 215-228). Liberation of this from the 2-chlorotrityl resin without removal of the side-chain protecting groups, activation of the carboxyl group and coupling to any amine-functionalized labeling group provides a di-lysine derivative whose protected pendant nitrogen atoms can be unmasked to give two free amino groups. The prior-mentioned safety-catch resin is activated and the desired N-deprotected labeling group-functionalized di-lysine derivative is added to the activated safety-catch resin. The pendant amino groups are acylated by the carboxy-terminus of the safety-catch resin-bound peptide, which is now detached from the resin and represents an integral part of the di-lysine structure. An excess of the safety-catch resin-bound peptide can be employed to insure complete reaction of the amino groups of the di-lysine construct. Optimization of the ratio of the reacting partners in this scheme optimizes the yield. The protecting groups on the cMet-binding peptides are removed employing trifluoroacetic acid based cleavage protocols.

The synthesis of dimeric and multimeric constructs wherein two or more cMet-binding peptides are present in one construct is easily accomplished. Orthogonal protection schemes (such as an allyloxycarbonyl group on one nitrogen and an Fmoc group on the other, or employing the Fmoc group in conjunction with the iV-Dde protecting group on the other, for example) can be employed to distinguish the pendant nitrogen atoms of the di-lysine derivatives described above. Unmasking of one of the amino groups, followed by reaction of the resulting product with an activated safety-catch resin-bound cMet-binding peptide as described above, provides a di-lysine construct having a single cMet-binding peptide attached. Removal of the second protecting group unmasks the remaining nitrogen (Mellor *et al.*, "Synthesis of Modified Peptides" in *Fmoc Solid Phase Peptide Synthesis*, Chan, W.C. and White, P.D., Eds. (Oxford University Press, New York, 2000), Chapt. 6, pp. 169-176). The resulting

product can be reacted with a second safety-catch resin bearing another cMet-binding peptide to provide a fully-protected homodimeric construct, which after removal of protecting groups with trifluoroacetic acid, provides the desired material.

Method B

A cMet-binding peptide is assembled on a Rink-amide resin by automated or manual peptide coupling methods, usually employing Fmoc peptide synthesis protocols. The peptide can possess a C-terminus or N-terminus functionalized with a linker or a linker-labeling group construct that may possess an additional nucleophilic group such as the ε-amino group of a lysine moiety, for example. Cleavage of the protecting groups is accomplished employing trifluoroacetic acid with appropriate modifiers depending on the nature of the peptide. The fully deprotected peptide is then reacted with a large excess of a bifunctional electrophile such as the commercially available glutaric acid bis-N-hydroxysuccinimide ester (Tyger Scientific, Inc., Princeton, NJ). The resulting monoamidated, mono-N-hydroxysuccinimidyl ester of glutaric acid is then treated with an additional equivalent of the same peptide, or an equivalent of a different cMet-binding peptide. Purification of the resulting material by HPLC affords the desired homo-dimeric construct bearing a suitable labeling group.

Method C

A modular scheme can be employed to prepare dimeric or higher multimeric constructs bearing suitable labeling groups as defined above. In a simple illustration, fmoc-lysine(iV-Dde) Rink amide resin is treated with piperidine to remove the fmoc moiety. Then a labeling function, such as biotin, 5-carboxyfluorescein or N,N-dimethyl-Gly-Ser(O-t-Bu)-Cys(Acm)-Gly-OH is coupled to the nitrogen atom. The resin is next treated with hydrazine to remove the iV-Dde group. After thorough washing, the resin is treated with cyanuric chloride and a hindered base such as diisopropylethylamine in a suitable solvent such as DMF, NMP or dichloromethane to provide a monofunctionalized dichlorotriazine bound to the resin. Subsequent successive displacement of the remaining chlorine atoms by two equivalents of a cMet-binding peptide provides a resin-bound homo-dimeric labeling group-functionalized

construct (Falorni, M. *et al.*, 1998. *Tetrahedron Lett.*, 39:7607-7610; Johnson, C. *et al.*, 1998. *Tetrahedron*, 54:4097-4106; Stankova, M. and Lebl, M., 1996. *Mol. Divers.*, 2:75-80). The incoming peptides can be protected or unprotected as the situation warrants. Cleavage of protecting groups is accomplished employing trifluoroacetic acid-based deprotection reagents as described above, and the desired materials are purified by high performance liquid chromatography.

It is understood that in each of these methods lysine derivatives can be serially employed to increase the multiplicity of the multimers. The use of related, more rigid molecules bearing the requisite number of masked, or orthogonally protected nitrogen atoms to act as scaffolds to vary the distance between the cMet-binding peptides, to increase the rigidity of the construct (by constraining the motion and relative positions of the cMet-binding peptides relative to each other and the reporter) is entirely within the scope of methods A-C and all other methods described herein. The references cited above are incorporated by reference herein in their entirety.

Uses for cMet Binding Polypeptides and Multimeric Peptide Constructs

The cMet binding moieties of the invention also have utility in the treatment of a variety of disease states, including those associated with cellular proliferation (*e.g.*, hyperproliferation, *e.g.*, cancer). The cMet binding moieties of the invention (*e.g.*, polypeptides and multimeric polypeptide constructs) can themselves be used as therapeutics or could be used to localize one or more therapeutic agents (*e.g.*, a chemotherapeutic, a radiotherapeutic, genetic material, etc.) to cMet-expressing cells, including sites of cellular proliferation. Any suitable method of assaying or imaging cMet also can be employed. The cMet binding moieties according to this invention are useful for detection and/or imaging of cMet *in vitro* or *in vivo*, and particularly for detection and/or imaging of sites of angiogenesis, in which HGF and cMet are intimately involved, as explained herein.

In vitro

For detection of HGF or cMet in solution, a binding polypeptide or multimeric polypeptide construct according to the invention can be detectably labeled, *e.g.*,

fluorescently labeled, enzymatically labeled, or labeled with a radioactive or paramagnetic metal, then contacted with the solution, and thereafter formation of a complex between the binding polypeptide and the cMet target can be detected. As an example, a fluorescently labeled cMet binding peptide can be used for *in vitro* cMet or HGF/cMet complex detection assays, wherein the peptide is added to a solution to be tested for cMet or HGF/cMet complex under conditions allowing binding to occur. The complex between the fluorescently labeled cMet binding peptide and cMet or HGF/cMet complex target can be detected and quantified by, for example, measuring the increased fluorescence polarization arising from the cMet or HGF/cMet complex-bound peptide relative to that of the free peptide.

Alternatively, a sandwich-type "ELISA" assay can be used, wherein a cMet binding polypeptide is immobilized on a solid support such as a plastic tube or well, then the solution suspected of containing cMet or HGF/cMet complex target is contacted with the immobilized binding moiety, non-binding materials are washed away, and complexed polypeptide is detected using a suitable detection reagent, such as a monoclonal antibody recognizing cMet or HGF/cMet complex. The monoclonal antibody is detectable by conventional means known in the art, including being detectably labeled, *e.g.*, radiolabeled, conjugated with an enzyme such as horseradish peroxidase and the like, or fluorescently labeled, etc.

For detection or purification of soluble cMet or HGF/cMet complex in or from a solution, binding polypeptides or multimeric polypeptide construct of the invention can be immobilized on a solid substrate such as a chromatographic support or other matrix material, then the immobilized binder can be loaded or contacted with the solution under conditions suitable for formation of a binding polypeptide/cMet complex. The non-binding portion of the solution can be removed and the complex can be detected, for example, using an anti-HGF or anti-HGF/cMet complex antibody, or an anti-binding polypeptide antibody, or the cMet or HGF/cMet complex target can be released from the binding moiety at appropriate elution conditions.

The biology of cellular proliferation and the roles of HGF and cMet in initiating and maintaining it have been investigated by many researchers and continues to be an active field for research and development. In furtherance of such research and

development, a method of purifying bulk amounts of cMet or HGF/cMet complex in pure form is desirable, and the binding polypeptides and multimeric polypeptide constructs according to this invention are especially useful for that purpose, using the general purification methodology described above.

In vivo

Diagnostic Imaging

A particularly preferred use for the polypeptides and multimeric polypeptide constructs according to the present invention is for creating visually readable images of cMet expressing tissue, such as, for example, neoplastic tumors, which exhibit hyperproliferation. The cMet binding polypeptides and multimeric polypeptide constructs disclosed herein can be converted to imaging reagents by conjugating the polypeptides with a label appropriate for diagnostic detection, optionally via a linker. Preferably, a peptide or multimeric polypeptide construct exhibiting much greater specificity for cMet or HGF/cMet than for other serum proteins is conjugated or linked to a label appropriate for the detection methodology to be employed. For example, the cMet or HGF/cMet complex binding polypeptide can be conjugated with or without a linker to a paramagnetic chelate suitable for Magnetic Resonance Imaging (MRI), with a radiolabel suitable for x-ray, Positron Emission Tomography (PET) or scintigraphic imaging (including a chelator for a radioactive metal), with an ultrasound contrast agent (*e.g.*, a stabilized microbubble, a microballoon, a microsphere or what has been referred to as a gas filled "liposome") suitable for ultrasound detection, or with an optical imaging dye.

Suitable linkers can include those discussed herein, including substituted or unsubstituted alkyl chains, amino acid chains (*e.g.*, polyglycine), polyethylene glycols, polyamides, and other linkers known in the art.

In general, the technique of using a detectably labeled cMet binding moiety is based on the premise that the label generates a signal that is detectable outside a patient's body. For example, when the detectably labeled cMet binding moiety is administered to the patient in which it is desirable to detect, *e.g.*, hyperproliferation, the high affinity of the cMet binding moiety for cMet causes the binding moiety to bind to

the site of hyperproliferation and accumulate label at the site. Sufficient time is allowed for the labeled binding moiety to localize at the site of proliferation. The signal generated by the labeled peptide is detected by a scanning device that will vary according to the type of label used, and the signal is then converted to an image of the site of proliferation.

In another embodiment, rather than directly labeling a cMet binding polypeptide or multimeric polypeptide construct with a detectable label or radiotherapeutic construct, one or more peptides or constructs of the invention can be conjugated with for example, avidin, biotin, or an antibody or antibody fragment that will bind the detectable label or radiotherapeutic. For example, one or more cMet-binding peptides can be conjugated to streptavidin (potentially generating multivalent binding) for *in vivo* binding to cMet-expressing cells. After the unbound targeting construct is cleared from the body, a biotinylated detectable label or radiotherapeutic construct (*e.g.*, a chelate molecule complexed with a radioactive metal) can be infused and will rapidly concentrate at the site where the targeting construct is bound. This approach in some situations can reduce the time required after administering the detectable label until imaging can take place. It also can increase signal to noise ratio in the target site, and decrease the dose of the detectable label or radiotherapeutic construct required. This is particularly useful when a radioactive label or radiotherapeutic is used as the dose of radiation that is delivered to normal but radiation-sensitive sites in the body, such as bone-marrow, kidneys, and liver is decreased. This approach, sometimes referred to as pre-targeting or two-step, or three-step approaches was reviewed by S.F. Rosebrough in *Q. J. Nucl. Med.*, 40:234-251 (1996), which is incorporated by reference herein.

A. Magnetic Resonance Imaging

The cMet binding moieties of the present invention can advantageously be conjugated with a paramagnetic metal chelate in order to form a contrast agent for use in MRI. Preferred paramagnetic metal ions have atomic numbers 21-29, 42, 44, or 57-83. This includes ions of the transition metal or lanthanide series which have one, and more preferably five or more, unpaired electrons and a magnetic moment of at least 1.7 Bohr magneton. Preferred paramagnetic metals include, but are not limited to,

chromium (III), manganese (II), manganese (III), iron (II), iron (III), cobalt (II), nickel (II), copper (II), praseodymium (III), neodymium (III), samarium (III), gadolinium (III), terbium (III), dysprosium (III), holmium (III), erbium (III), europium (III) and ytterbium (III), chromium (III), iron (III), and gadolinium (III). The trivalent cation, Gd^{3+}, is particularly preferred for MRI contrast agents, due to its high relaxivity and low toxicity, with the further advantage that it exists in only one biologically accessible oxidation state, which minimizes undesired metabolysis of the metal by a patient. Another useful metal is Cr^{3+}, which is relatively inexpensive. Gd(III) chelates have been used for clinical and radiologic MR applications since 1988, and approximately 30% of MR exams currently employ a gadolinium-based contrast agent.

The practitioner will select a metal according to dose required to detect cellular proliferation and considering other factors such as toxicity of the metal to the subject. See, Tweedle et al., Magnetic Resonance Imaging (2nd ed.), vol. 1, Partain *et al.*, Eds. (W.B. Saunders Co. 1988), pp. 796-797. Generally, the desired dose for an individual metal will be proportional to its relaxivity, modified by the biodistribution, pharmacokinetics and metabolism of the metal.

The paramagnetic metal chelator is a molecule having one or more polar groups that act as a ligand for, and complex with, a paramagnetic metal. Suitable chelators are known in the art and include acids with methylene phosphonic acid groups, methylene carbohydroxamine acid groups, carboxyethylidene groups, or carboxymethylene groups. Examples of chelators include, but are not limited to, diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-tetraazacyclo-tetradecane-1,4,7,10-tetraacetic acid (DOTA), 1-substituted 1,4,7,-tricarboxymethyl-1,4,7,10-teraazacyclododecane (DO3A), ethylenediaminetetraacetic acid (EDTA), and 1,4,8,11-tetra-azacyclotetradecane-1,4,8,11-tetraacetic acid (TETA). Additional chelating ligands are ethylene bis-(2-hydroxy-phenylglycine) (EHPG), and derivatives thereof, including 5-Cl-EHPG, 5-Br-EHPG, 5-Me-EHPG, 5-t-Bu-EHPG, and 5-sec-Bu-EHPG; benzodiethylenetriamine pentaacetic acid (benzo-DTPA) and derivatives thereof, including dibenzo-DTPA, phenyl-DTPA, diphenyl-DTPA, benzyl-DTPA, and dibenzyl DTPA; bis-2 (hydroxybenzyl)-ethylene-diaminediacetic acid (HBED) and derivatives thereof; the class of macrocyclic compounds which contain at least 3 carbon atoms,

more preferably at least 6, and at least two heteroatoms (O and/or N), which macrocyclic compounds can consist of one ring, or two or three rings joined together at the hetero ring elements, *e.g.*, benzo-DOTA, dibenzo-DOTA, and benzo-NOTA, where NOTA is 1,4,7-triazacyclononane N,N',N"-triacetic acid, benzo-TETA, benzo-DOTMA, where DOTMA is 1,4,7,10-tetraazacyclotetradecane-1,4,7, 10-tetra(methyl tetraacetic acid), and benzo-TETMA, where TETMA is 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-(methyl tetraacetic acid); derivatives of 1,3-propylene-diaminetetraacetic acid (PDTA) and triethylenetetraaminehexaacetic acid (TTHA); derivatives of 1,5,10?N,N',N"-tris(2,3-dihydroxybenzoyl)-tricatecholate (LICAM); and 1,3,5-N,N',N"-tris(2,3-dihydroxybenzoyl) aminomethylbenzene (MECAM). A preferred chelator for use in the present invention is DTPA, and the use of DO3A is particularly preferred. Examples of representative chelators and chelating groups contemplated by the present invention are described in WO 98/18496, WO 86/06605, WO 91/03200, WO 95/28179, WO 96/23526, WO 97/36619, PCT/US98/01473, PCT/US98/20182, and US 4,899,755, US 5,474,756, US 5,846,519 and US 6,143,274, all of which are hereby incorporated by reference.

In accordance with the present invention, the chelator of the MRI contrast agent is coupled to the cMet binding polypeptide. The positioning of the chelate should be selected so as not to interfere with the binding affinity or specificity of the cMet binding polypeptide. Preferably, the chelate will be appended either to the N-terminus or the C-terminus, however the chelate also can be attached anywhere within the sequence. In preferred embodiments, a chelator having a free central carboxylic acid group (*e.g.*, DTPA-Asp(β-COOH)-)OtBu) makes it easy to attach at the N-terminus of the peptide by formation of an amide bond. The chelate also can be attached at the C-terminus with the aid of a linker. Alternatively, isothiocyanate conjugation chemistry can be employed as a way of linking the appropriate isothiocyanate group bearing DTPA to a free amino group anywhere within the peptide sequence.

In general, the cMet binding moiety can be bound directly or covalently to the metal chelator (or other detectable label), or it can be coupled or conjugated to the metal chelator using a linker, which can be, without limitation, amide, urea, acetal, ketal, double ester, carbonyl, carbamate, thiourea, sulfone, thioester, ester, ether, disulfide,

lactone, imine, phosphoryl, or phosphodiester linkages; substituted or unsubstituted saturated or unsaturated alkyl chains; linear, branched, or cyclic amino acid chains of a single amino acid or different amino acids (*e.g.*, extensions of the N- or C- terminus of the cMet binding moiety); derivatized or underivatized polyethylene glycols (PEGs), polyoxyethylene, or polyvinylpyridine chains; substituted or unsubstituted polyamide chains; derivatized or underivatized polyamine, polyester, polyethylenimine, polyacrylate, poly(vinyl alcohol), polyglycerol, or oligosaccharide (*e.g.*, dextran) chains; alternating block copolymers; malonic, succinic, glutaric, adipic and pimelic acids; caproic acid; simple diamines and dialcohols; any of the other linkers disclosed herein; or any other simple polymeric linkers known in the art (see, for example, WO 98/18497 and WO 98/18496). Preferably the molecular weight of the linker can be tightly controlled. The molecular weights can range in size from less than 100 to greater than 1000. Preferably the molecular weight of the linker is less than 100. In addition, it can be desirable to utilize a linker that is biodegradable *in vivo* to provide efficient routes of excretion for the imaging reagents of the present invention. Depending on their location within the linker, such biodegradable functionalities can include ester, double ester, amide, phosphoester, ether, acetal, and ketal functionalities.

In general, known methods can be used to couple the metal chelate and the cMet binding moiety using such linkers (WO 95/28967, WO 98/18496, WO 98/18497 and discussion therein). The cMet binding moiety can be linked through an N- or C-terminus via an amide bond, for example, to a metal coordinating backbone nitrogen of a metal chelate or to an acetate arm of the metal chelate itself. The present invention contemplates linking of the chelate on any position, provided the metal chelate retains the ability to bind the metal tightly in order to minimize toxicity. Similarly, the cMet binding moiety can be modified or elongated in order to generate a locus for attachment to a metal chelate, provided such modification or elongation does not eliminate its ability to bind cMet.

MRI contrast reagents prepared according to the disclosures herein can be used in the same manner as conventional MRI contrast reagents. When imaging a site of hyperproliferation, for example, certain MR techniques and pulse sequences can be preferred to enhance the contrast of the site to the background blood and tissues. These

techniques include (but are not limited to), for example, black blood angiography sequences that seek to make blood dark, such as fast spin echo sequences (Alexander, A. *et al.*, 1998. *Magn. Reson. Med.*, 40: 298-310) and flow-spoiled gradient echo sequences (Edelman, R. *et al.*, 1990. *Radiology*, 177: 45-50). These methods also include flow independent techniques that enhance the difference in contrast, such as inversion-recovery prepared or saturation-recovery prepared sequences that will increase the contrast between angiogenic tumor and background tissues. Finally, magnetization transfer preparations also can improve contrast with these agents (Goodrich, K. *et al.*, 1996. *Invest. Radiol.*, 31: 323-32).

The labeled reagent is administered to the patient in the form of an injectable composition. The method of administering the MRI contrast agent is preferably parenterally, meaning intravenously, intraarterially, intrathecally, interstitially, or intracavitarilly. For imaging active angiogenesis, intravenous or intraarterial administration is preferred. For MRI, it is contemplated that the subject will receive a dosage of contrast agent sufficient to enhance the MR signal at the site of angiogenesis at least 10%. After injection with the cMet binding moiety-containing MRI reagent, the patient is scanned in the MRI machine to determine the location of any sites of hyperproliferation. In therapeutic settings, upon identification of a site of hyperproliferation (*e.g.*, tumor), a tumoricidal agent or anti-hyperproliferative agent (*e.g.*, inhibitors of HGF) can be immediately administered, if necessary, and the patient can be subsequently scanned to visualize tumor regression or arrest of angiogenesis.

B. Ultrasound Imaging

When ultrasound is transmitted through a substance, the acoustic properties of the substance will depend upon the velocity of the transmissions and the density of the substance. Changes in the acoustic properties will be most prominent at the interface of different substances (solids, liquids, gases). Ultrasound contrast agents are intense sound wave reflectors because of the acoustic differences between the agent and the surrounding tissue. Gas containing or gas generating ultrasound contrast agents are particularly useful because of the acoustic difference between liquid (*e.g.*, blood) and the gas-containing or gas generating ultrasound contrast agent. Because of their size,

ultrasound contrast agents comprising microbubbles, microballoons, and the like can remain for a longer time in the blood stream after injection than other detectable moieties; a targeted cMet-specific ultrasound agent therefore could demonstrate superior imaging of sites of hyperproliferation (*e.g.*, cancer) and angiogenesis.

In this aspect of the invention, the cMet binding moiety can be linked to a material that is useful for ultrasound imaging. For example, one or more cMet binding polypeptide or multimeric polypeptide constructs can be linked to materials employed to form vesicles (*e.g.*, microbubbles, microballoons, microspheres, etc.), or emulsions containing a liquid or gas, which functions as the detectable label (*e.g.*, an echogenic gas or material capable of generating an echogenic gas). Materials for the preparation of such vesicles include surfactants, lipids, sphingolipids, oligolipids, phospholipids, proteins, polypeptides, carbohydrates, and synthetic or natural polymeric materials (WO 98/53857, WO 98/18498, WO 98/18495, WO 98/18497, WO 98/18496, and WO 98/18501, incorporated herein by reference in their entirety).

For contrast agents comprising suspensions of stabilized microbubbles (a preferred embodiment), phospholipids, and particularly saturated phospholipids are preferred. Examples of suitable phospholipids include esters of glycerol with one or two (the same or different) fatty acids molecules and with phosphoric acid, wherein the phosphoric acid residue is in turn bonded to a hydrophilic group, such as choline, serine, inositol, glycerol, ethanolamine, and the like groups. Fatty acids present in the phospholipids are in general long chain aliphatic acids, typically containing from 12 to 24 carbon atoms, preferably from 14 to 22, that can be saturated or can contain one or more unsaturations. Examples of suitable fatty acids are lauric acid, myristic acid, palmitic acid, stearic acid, arachidonic acid, behenic acid, oleic acid, linoleic acid, and linolenic acid. Mono esters of phospholipid are also known in the art as the "lyso" forms of the phospholipids. Further examples of phospholipid are phosphatidic acids, *i.e.*, the diesters of glycerol-phosphoric acid with fatty acids, sphingomyelins, *i.e.*, those phosphatidylcholine analogs where the residue of glycerol diester with fatty acids is replaced by a ceramide chain, cardiolipins, *i.e.*, the esters of 1,3-diphosphatidylglycerol with a fatty acid, gangliosides, cerebrosides, etc.

As used herein, the term "phospholipids" includes naturally occurring, semisynthetic or synthetically prepared products that can be employed either singularly or as mixtures.

Examples of naturally occurring phospholipids are natural lecithins (phosphatidylcholine (PC) derivatives) such as, typically, soya bean or egg yolk lecithins. Examples of semisynthetic phospholipids are the partially or fully hydrogenated derivatives of the naturally occurring lecithins.

Examples of synthetic phospholipids are, *e.g.*, dilauryloyl-phosphatidylcholine ("DLPC"), dimyristoylphosphatidylcholine ("DMPC"), dipalmitoyl-phosphatidylcholine ("DPPC"), diarachidoylphosphatidylcholine ("DAPC"), distearoyl-phosphatidylcholine ("DSPC"), 1-myristoyl-2-palmitoylphosphatidylcholine ("MPPC"), 1-palmitoyl-2-myristoylphosphatidylcholine ("PMPC"), 1-palmitoyl-2-stearoylphosphatid-ylcholine ("PSPC"), 1-stearoyl-2-palmitoyl-phosphatidylcholine ("SPPC"), dioleoylphosphatidylycholine ("DOPC"), 1,2 Distearoyl-sn-glycero-3-Ethylphosphocholine (Ethyl-DSPC), dilauryloyl-phosphatidylglycerol ("DLPG") and its alkali metal salts, diarachidoylphosphatidylglycerol ("DAPG") and its alkali metal salts, dimyristoylphosphatidylglycerol ("DMPG") and its alkali metal salts, dipalmitoyl-phosphatidylglycerol ("DPPG") and its alkali metal salts, distearolyphosphatidylglycerol ("DSPG") and its alkali metal salts, dioleoylphosphatidylglycerol ("DOPG") and its alkali metal salts, dimyristoyl phosphatidic acid ("DMPA") and its alkali metal salts, dipalmitoyl phosphatidic acid ("DPPA") and its alkali metal salts, distearoyl phosphatidic acid ("DSPA"), diarachidoyl phosphatidic acid ("DAPA") and its alkali metal salts, dimyristoyl phosphatidyl-ethanolamine ("DMPE"), dipalmitoyl phosphatidylethanolamine ("DPPE"), distearoyl phosphatidyl-ethanolamine ("DSPE"), dimyristoyl phosphatidylserine ("DMPS"), diarachidoyl phosphatidylserine ("DAPS"), dipalmitoyl phosphatidylserine ("DPPS"), distearoylphosphatidylserine ("DSPS"), dioleoylphosphatidylserine ("DOPS"), dipalmitoyl sphingomyelin ("DPSP"), and distearoyl sphingomyelin ("DSSP"). In a preferred embodiment, at least one of the phospholipid moieties has the structure shown in FIGS. 7A or 7B, and described in US 5,686,060, which is herein incorporated by reference.

Other preferred phospholipids include dipalmitoylphosphatidylcholine, dipalmitoylphosphatidic acid and dipalmitoylphosphatidylserine. The compositions also can contain PEG-4000 and/or palmitic acid. Any of the gases disclosed herein or known to the skilled artisan can be employed; however, inert gases, such as SF6 or fluorocarbons like CF4, C3F8 and C4F10, are preferred.

The preferred gas-filled microbubbles of the invention can be prepared by means known in the art, such as, for example, by a method described in any one of the following patents: EP 554213, US 5,413,774, US 5,578,292, EP 744962, EP 682530, US 5,556,610, US 5,846,518, US 6,183,725, EP 474833, US 5,271,928, US 5,380,519, US 5,531,980, US 5,567,414, US 5,658,551, US 5,643,553, US 5,911,972, US 6,110,443, US 6,136,293, EP 619743, US 5,445,813, US 5,597,549, US 5,686,060, US 6,187,288, and US 5,908,610, which are incorporated by reference herein in their entirety.

The preferred microbubble suspensions of the present invention can be prepared from phospholipids using known processes such as a freeze-drying or spray-drying solutions of the crude phospholipids in a suitable solvent or using the processes set forth in EP 554213; US 5,413,774; US 5,578,292; EP 744962; EP 682530; US 5,556,610; US 5,846,518; US 6,183,725; EP 474833; US 5,271,928; US 5,380,519; US 5,531,980; US 5,567,414; US 5,658,551; US 5,643,553; US 5,911,972; US 6,110,443; US 6,136,293; EP 619743; US 5,445,813; US 5,597,549; US 5,686,060; US 6,187,288; and US 5,908,610; which are incorporated by reference herein in their entirety. Most preferably, the phospholipids are dissolved in an organic solvent and the solution is dried without going through a liposome formation stage. This can be done by dissolving the phospholipids in a suitable organic solvent together with a hydrophilic stabilizer substance or a compound soluble both in the organic solvent and water and freeze-drying or spray-drying the solution. In this embodiment the criteria used for selection of the hydrophilic stabilizer is its solubility in the organic solvent of choice. Examples of hydrophilic stabilizer compounds soluble in water and the organic solvent are, *e.g.*, a polymer, like polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyethylene glycol (PEG), etc., malic acid, glycolic acid, maltol, and the like. Such hydrophilic compounds also aid in homogenizing the microbubbles size distribution and

enhance stability under storage. Any suitable organic solvent can be used as long as its boiling point is sufficiently low and its melting point is sufficiently high to facilitate subsequent drying. Typical organic solvents include, for example, dioxane, cyclohexanol, tertiary butanol, tetrachlorodifluoro ethylene ($C_2Cl_4F_2$) or 2-methyl-2-butanol. 2-methyl-2-butanol and $C_2Cl_4F_2$ are preferred.

Prior to formation of the suspension of microbubbles by dispersion in an aqueous carrier, the freeze dried or spray dried phospholipid powders are contacted with air or another gas. When contacted with the aqueous carrier the powdered phospholipids whose structure has been disrupted will form lamellarized or laminarized segments that will stabilize the microbubbles of the gas dispersed therein. This method permits production of suspensions of microbubbles, which are stable even when stored for prolonged periods, and are obtained by simple dissolution of the dried laminarized phospholipids, which have been stored under a desired gas, without shaking or any violent agitation.

Unless it contains a hyperpolarized gas, known to require special storage conditions, the lyophilized or freeze-dried residue can be stored and transported without need of temperature control of its environment and in particular it can be supplied to hospitals and physicians for on site formulation into a ready-to-use administrable suspension without requiring such users to have special storage facilities.

Preferably in such a case it can be supplied in the form of a two component kit. The two component kit can include two separate containers or a dual-chamber container. In the former case preferably the container is a conventional septum-sealed vial, wherein the vial containing the lyophilized residue of step b) is sealed with a septum through which the carrier liquid can be injected using an optionally pre-filled syringe. In such a case the syringe used as the container of the second component is also used then for injecting the contrast agent. In the latter case, preferably the dual-chamber container is a dual-chamber syringe and once the lyophilizate/freeze-dried residue has been reconstituted and then suitably mixed or gently shaken, the container can be used directly for injecting the contrast agent. In both cases means for directing or permitting application of sufficient bubble forming energy into the contents of the container are provided. However, as noted above, in the stabilized contrast agents the

size of the gas microbubbles is substantially independent of the amount of agitation energy applied to the reconstituted dried product. Accordingly no more than gentle hand shaking is generally required to give reproducible products with consistent microbubble size.

It can be appreciated by one ordinary skilled in the art that other two-chamber reconstitution systems capable of combining the dried powder with the aqueous solution in a sterile manner are also within the scope of the present invention. In such systems, it is particularly advantageous if the aqueous phase can be interposed between the water-insoluble gas and the environment, to increase shelf life of the product. Where a material necessary for forming the contrast agent is not already present in the container (*e.g.*, a cMet binding moiety of the invention to be linked to the phospholipid during reconstitution), it can be packaged with the other components of the kit, preferably in a form or container adapted to facilitate ready combination with the other components of the kit.

No specific containers, vial or connection systems are required; the present invention can use conventional containers, vials and adapters. The only requirement is a good seal between the stopper and the container. The quality of the seal, therefore, becomes a matter of primary concern; any degradation of seal integrity could allow undesirables substances to enter the vial. In addition to assuring sterility, vacuum retention is essential for products stoppered at ambient or reduced pressures to assure safe and proper reconstitution. As to the stopper, it may be a compound or multicomponent formulation based on an elastomer, such as poly(isobutylene) or butyl rubber.

Alternatively, microbubbles can be prepared by suspending a gas in an aqueous solution at high agitation speed, as disclosed, *e.g.*, in WO 97/29783. A further process for preparing microbubbles is disclosed in co-pending European patent application no. 03002373, herein incorporated by reference, which comprises preparing an emulsion of an organic solvent in an aqueous medium in the presence of a phospholipid and subsequently lyophilizing said emulsion, after optional washing and/or filtration steps.

Additives known to those of ordinary skill in the art can be included in the suspensions of stabilized microbubbles. For instance, non-film forming surfactants,

including polyoxypropylene glycol and polyoxyethylene glycol and similar compounds, as well as various copolymers thereof; fatty acids such as myristic acid, palmitic acid, stearic acid, arachidonic acid or their derivatives, ergosterol, phytosterol, sitosterol, lanosterol, tocopherol, propyl gallate, ascorbyl palmitate and butylated hydroxytoluene may be added. The amount of these non-film forming surfactants is usually up to 50% by weight of the total amount of surfactants but preferably between 0 and 30%.

In ultrasound applications the contrast agents formed by phospholipid stabilized microbubbles can, for example, be administered in doses such that the amount of phospholipid injected is in the range 0.1 to 200 μg/kg body weight, preferably from about 0.1 to 30 μg/kg.

Other gas containing suspensions include those disclosed in, for example, US 5,798,091, WO 97/29783, also EP 881 915, incorporated herein by reference in their entirety. These agents can be prepared as described in US 5,798,091 or WO97/29783.

Another preferred ultrasound contrast agent comprises microballoons. The term "microballoon" refers to gas filled bodies with a material boundary or envelope. More on microballoon formulations and methods of preparation can be found in EP 324 938 (US 4,844,882); US 5,711,933; US 5,840,275; US 5,863,520; US 6,123,922; US 6,200,548; US 4,900,540; US 5,123,414; US 5,230,882; US 5,469,854; US 5,585,112; US 4,718,433; US 4,774,958; WO 95/01187; US 5,529,766; US 5,536,490; and US 5,990,263, the contents of which are incorporated herein by reference.

The preferred microballoons have an envelope including a biodegradable physiologically compatible polymer or, a biodegradable solid lipid. The polymers useful for the preparation of the microballoons of the present invention can be selected from the biodegradable physiologically compatible polymers, such as any of those described in any of the following patents: EP 458745; US 5,711,933; US 5,840,275; EP 554213; US 5,413,774; and US 5,578,292, the entire contents of which are incorporated herein by reference. In particular, the polymer can be selected from biodegradable physiologically compatible polymers, such as polysaccharides of low water solubility, polylactides and polyglycolides and their copolymers, copolymers of lactides and lactones such as e-caprolactone, γ-valerolactone and polypeptides. Other suitable polymers include poly(ortho)esters (see for instance US 4,093,709; US 4,131,648; US

4,138,344; US 4,180,646); polylactic and polyglycolic acid and their copolymers, for instance DEXON (Heller, J., 1980. *Biomaterials*, 1:51-57); poly(DL-lactide-co-e-caprolactone), poly(DL-lactide-co-γ-valerolactone), poly(DL-lactide-co-γ-butyrolactone), polyalkylcyanoacrylates; polyamides, polyhydroxybutyrate; polydioxanone; poly-β-aminoketones (*Polymer*, 23:1693 (1982)); polyphosphazenes (Allcock, H., 1976. *Science*, 193:1214-1219); and polyanhydrides. The microballoons of the present invention can also be prepared according to the methods of WO 96/15815, incorporated herein by reference, where the microballoons are made from a biodegradable membrane comprising biodegradable lipids, preferably selected from mono- di-, tri-glycerides, fatty acids, sterols, waxes and mixtures thereof. Preferred lipids are di- or tri-glycerides, e.g. di- or tri-myristin, -palmityn or -stearin, in particular tripalmitin or tristearin.

The microballoons can employ any of the gases disclosed herein of known to the skilled artisan; however, inert gases such as fluorinated gases are preferred. The microballoons can be suspended in a pharmaceutically acceptable liquid carrier with optional additives known to those of ordinary skill in the art and stabilizers.

Microballoons-containing contrast agents are typically administered in doses such that the amount of wall-forming polymer or lipid is from about 10 μg/kg to about 20 μg/kg of body weight.

Other gas-containing contrast agent formulations include microparticles (especially aggregates of microparticles) having gas contained therein or otherwise associated therewith (for example being adsorbed on the surface thereof and/or contained within voids, cavities or pores therein). Methods for the preparation of these agents are as described in EP 0122624; EP 0123235; EP 0365467; US 5,558,857; US 5,607,661; US 5,637,289; US 5,558,856; US 5,137,928; WO 95/21631 or WO 93/13809, incorporated herein by reference in their entirety.

Any of these ultrasound compositions also should be, as far as possible, isotonic with blood. Hence, before injection, small amounts of isotonic agents can be added to any of above ultrasound contrast agent suspensions. The isotonic agents are physiological solutions commonly used in medicine and they comprise aqueous saline solution (0.9% NaCl), 2.6% glycerol solution, 5% dextrose solution, etc. Additionally,

the ultrasound compositions can include standard pharmaceutically acceptable additives, including, for example, emulsifying agents, viscosity modifiers, cryoprotectants, lyoprotectants, bulking agents etc.

Any biocompatible gas can be used in the ultrasound contrast agents useful in the invention. The term "gas" as used herein includes any substances (including mixtures) substantially in gaseous form at the normal human body temperature. The gas may thus include, for example, air, nitrogen, oxygen, CO_2, argon, xenon or krypton, fluorinated gases (including for example, perfluorocarbons, SF_6, SeF_6) a low molecular weight hydrocarbon (*e.g.*, containing from 1 to 7 carbon atoms), for example, an alkane such as methane, ethane, a propane, a butane or a pentane, a cycloalkane such as cyclopropane, cyclobutane or cyclopentene, an alkene such as ethylene, propene, propadiene or a butene, or an alkyne such as acetylene or propyne and/or mixtures thereof. However, fluorinated gases are preferred. Fluorinated gases include materials which contain at least one fluorine atom such as SF_6, freons (organic compounds containing one or more carbon atoms and fluorine, *i.e.*, CF_4, C_2F_6, C_3F_8, C_4F_8,C_4F_{10},$CBrF_3$, CCl_2F_2,C_2ClF_5, and $CBrClF_2$) and perfluorocarbons. The term perfluorocarbon refers to compounds containing only carbon and fluorine atoms and includes, in particular, saturated, unsaturated, and cyclic perfluorocarbons. The saturated perfluorocarbons, which are usually preferred, have the formula C_nF_{n+2}, where n is from 1 to 12, preferably from 2 to 10, most preferably from 3 to 8 and even more preferably from 3 to 6. Suitable perfluorocarbons include, for example, CF_4, C_2F_6, C_3F_8 C_4F_8, C_4F_{10}, C_5F_{12}, C_6F_{12}, C_7F_{14}, C_8F_{18}, and C_9F_{20}. Most preferably the gas or gas mixture comprises SF6 or a perfluorocarbon selected from the group consisting of C_3F_8 C_4F_8, C_4F_{10}, C_5F_{12}, C_6F_{12}, C_7F_{14}, C_8F_{18}, with C_4F_{10} being particularly preferred. See also WO 97/29783, WO 98/53857, WO 98/18498, WO 98/18495, WO 98/18496, WO 98/18497, WO 98/18501, WO 98/05364, WO 98/17324.

In certain circumstances it can be desirable to include a precursor to a gaseous substance (*e.g.*, a material that is capable of being converted to a gas *in vivo*, often referred to as a "gas precursor"). Preferably the gas precursor and the gas it produces are physiologically acceptable. The gas precursor can be pH-activated, photo-activated, temperature activated, etc. For example, certain perfluorocarbons can be used as

temperature activated gas precursors. These perfluorocarbons, such as perfluoropentane, have a liquid/gas phase transition temperature above room temperature (or the temperature at which the agents are produced and/or stored) but below body temperature; thus they undergo a phase shift and are converted to a gas within the human body.

The gas can comprise a mixture of gases. The following combinations are particularly preferred gas mixtures: a mixture of gases (A) and (B) in which, at least one of the gases (B), present in an amount of between 0.5 - 41% by vol., has a molecular weight greater than 80 daltons and is a fluorinated gas and (A) is selected from the group consisting of air, oxygen, nitrogen, carbon dioxide and mixtures thereof, the balance of the mixture being gas A.

Since ultrasound vesicles can be larger than the other detectable labels described herein, they can be linked or conjugated to a plurality of cMet binding polypeptides or multimeric polypeptide constructs in order to increase the targeting efficiency of the agent. Attachment to the ultrasound contrast agents described above (or known to those skilled in the art) can be via direct covalent bond between the cMet binding polypeptide and the material used to make the vesicle or via a linker, as described previously.[1] For example, see WO 98/53857 generally for a description of the attachment of a peptide to a bifunctional PEG linker, which is then reacted with a liposome composition (Lanza, G. *et al.*, 1997. *Ultrasound Med. Biol.*, 23:863-870).). The structure of these compounds typically comprises:

a) A hydrophobic portion, compatible with the material forming the envelope of the microbubble or of the microballoon, in order to allow an effective incorporation of the compound in the envelope of the vesicle; said portion is typically a lipid moiety (*e.g.*, dipalmitin, distearoil);

b) A spacer (typically PEGs of different molecular weights), which can be optional in some cases (microbubbles may, for instance, prove difficult to freeze dry if the spacer is too long) or preferred in some others (*e.g.*, peptides can be less active when conjugated to a microballoon with a short spacer);

c) A reactive group capable of reacting with a corresponding reactive moiety on the peptide to be conjugated (e.g., maleimido with the -SH group of cysteine).

A number of methods can be used to prepare suspensions of microbubbles conjugated to cMet binding polypeptides. For example, one can prepare maleimide-derivatized microbubbles by incorporating 5 % (w/w) of N-MPB-PE (1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-4-(p-maleimido-phenyl butyramide), (Avanti Polar-Lipids, Inc., Alabaster, AL) in the phospholipid formulation. Then, solutions of mercaptoacetylated cMet-binding peptides (10 mg/mL in DMF), which have been incubated in deacetylation solution (50 mM sodium phosphate, 25 mM EDTA, 0.5 M hydroxylamine.HCl, pH 7.5) are added to the maleimide-activated microbubble suspension. After incubation in the dark, under gentle agitation, the peptide conjugated microbubbles can be purified by centrifugation.

Alternatively, cMet-binding polypeptide conjugated microbubbles can be prepared using biotin/avidin. For example, avidin-conjugated microbubbles can be prepared using a maleimide-activated phospholipid microbubble suspension, prepared as described above, which is added to mercaptoacetylated-avidin (which has been incubated with deacetylation solution). Biotinylated cMet-binding peptides (prepared as described herein) are then added to the suspension of avidin-conjugated microbubbles, yielding a suspension of microbubbles conjugated to cMet-binding peptides.

Ultrasound imaging techniques, which can be used in accordance with the present invention, include known techniques, such as color Doppler, power Doppler, Doppler amplitude, stimulated acoustic imaging, and two- or three-dimensional imaging techniques. Imaging may be done in harmonic (resonant frequency) or fundamental modes, with the second harmonic preferred.

C. Optical Imaging, Sonoluminescence or Photoacoustic Imaging

In accordance with the present invention, a number of optical parameters can be employed to determine the location of cMet or HGF/cMet complex with *in vivo* light imaging after injection of the subject with an optically-labeled cMet binding polypeptides. Optical parameters to be detected in the preparation of an image may include transmitted radiation, absorption, fluorescent or phosphorescent emission, light

reflection, changes in absorbance amplitude or maxima, and elastically scattered radiation. For example, biological tissue is relatively translucent to light in the near infrared (NIR) wavelength range of 650-1000 nm. NIR radiation can penetrate tissue up to several centimeters, permitting the use of the cMet binding polypeptides or multimeric polypeptide constructs of the present invention for optical imaging of cMet or HGF/cMet complex *in vivo*.

The cMet binding polypeptides or multimeric polypeptide constructs can be conjugated with photolabels, such as, for example, optical dyes, including organic chromophores or fluorophores, having extensive delocalized ring systems and having absorption or emission maxima in the range of 400-1500 nm. The cMet binding polypeptide or multimeric polypeptide construct can alternatively be derivatized with a bioluminescent molecule. The preferred range of absorption maxima for photolabels is between 600 and 1000 nm to minimize interference with the signal from hemoglobin. Preferably, photoabsorption labels have large molar absorptivities, *e.g.*, greater than 10^5 $cm^{-1}M^{-1}$, while fluorescent optical dyes will have high quantum yields. Examples of optical dyes include, but are not limited to those described in WO 98/18497, WO 98/18496, WO 98/18495, WO 98/18498, WO 98/53857, WO 96/17628, WO 97/18841, WO 96/23524, WO 98/47538, and references cited therein. The photolabels can be covalently linked directly to the cMet binding peptide or linked to the cMet binding peptide or multimeric polypeptide construct via a linker, as described previously.

After injection of the optically-labeled cMet binding moiety, the patient is scanned with one or more light sources (*e.g.*, a laser) in the wavelength range appropriate for the photolabel employed in the agent. The light used can be monochromatic or polychromatic and continuous or pulsed. Transmitted, scattered, or reflected light is detected via a photodetector tuned to one or multiple wavelengths to determine the location of cMet or HGF/cMet complex in the subject. Changes in the optical parameter can be monitored over time to detect accumulation of the optically-labeled reagent at the site of hyperproliferation. Standard image processing and detecting devices can be used in conjunction with the optical imaging reagents of the present invention.

The optical imaging reagents described above also can be used for acousto-optical or sonoluminescent imaging performed with optically-labeled imaging agents (see, US 5,171,298, WO 98/57666, and references cited therein). In acousto-optical imaging, ultrasound radiation is applied to the subject and affects the optical parameters of the transmitted, emitted, or reflected light. In sonoluminescent imaging, the applied ultrasound actually generates the light detected. Suitable imaging methods using such techniques are described in WO 98/57666.

D. Nuclear Imaging (Radionuclide Imaging) and Radiotherapy

The cMet binding moieties can be conjugated with a radionuclide reporter appropriate for scintigraphy, SPECT, or PET imaging and/or with a radionuclide appropriate for radiotherapy. Constructs in which the cMet binding moieties are conjugated with both a chelator for a radionuclide useful for diagnostic imaging and a chelator useful for radiotherapy are within the scope of the invention.

For use as a PET agent a peptide or multimeric polypeptide construct is complexed with one of the various positron emitting metal ions, such as ^{51}Mn, ^{52}Fe, ^{60}Cu, ^{68}Ga, ^{72}As, ^{94m}Tc, or ^{110}In. The binding moieties of the invention can also be labeled by halogenation using radionuclides such as ^{18}F, ^{124}I, ^{125}I, ^{131}I, ^{123}I, ^{77}Br, and ^{76}Br. Preferred metal radionuclides for scintigraphy or radiotherapy include ^{99m}Tc, ^{51}Cr, ^{67}Ga, ^{68}Ga, ^{47}Sc, ^{51}Cr, ^{167}Tm, ^{141}Ce, ^{111}In, ^{168}Yb, ^{175}Yb, ^{140}La, ^{90}Y, ^{88}Y, ^{153}Sm, ^{166}Ho, ^{165}Dy, ^{166}Dy, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{97}Ru, ^{103}Ru, ^{186}Re, ^{188}Re, ^{203}Pb, ^{211}Bi, ^{212}Bi, ^{213}Bi, ^{214}Bi, ^{105}Rh, ^{109}Pd, ^{117m}Sn, ^{149}Pm, ^{161}Tb, ^{177}Lu, ^{198}Au and ^{199}Au. The choice of metal will be determined based on the desired therapeutic or diagnostic application. For example, for diagnostic purposes the preferred radionuclides include ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{99m}Tc, and ^{111}In. For therapeutic purposes, the preferred radionuclides include ^{64}Cu, ^{90}Y, ^{105}Rh, ^{111}In, ^{117m}Sn, ^{149}Pm, ^{153}Sm, ^{161}Tb, ^{166}Dy, ^{166}Ho, ^{175}Yb, ^{177}Lu, $^{186/188}Re$, and ^{199}Au. ^{99m}Tc is particularly preferred for diagnostic applications because of its low cost, availability, imaging properties, and high specific activity. The nuclear and radioactive properties of ^{99m}Tc make this isotope an ideal scintigraphic imaging agent. This isotope has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ^{99}Mo-^{99m}Tc generator.

The metal radionuclides may be chelated by, for example, linear, macrocyclic, terpyridine, and N_3S, N_2S_2, or N_4 chelants (see also, US 5,367,080, US 5,364,613, US 5,021,556, US 5,075,099, US 5,886,142), and other chelators known in the art including, but not limited to, HYNIC, DTPA, EDTA, DOTA, DO3A, TETA, and bisamino bisthiol (BAT) chelators (see also US 5,720,934). For example, N_4 chelators are described in US 6,143,274; US 6,093,382; US 5,608,110; US 5,665,329; US 5,656,254; and US 5,688,487. Certain N_3S chelators are described in PCT/CA94/00395, PCT/CA94/00479, PCT/CA95/00249 and in US5,662,885; US 5,976,495; and US 5,780,006. The chelator also can include derivatives of the chelating ligand mercapto-acetyl-acetyl-glycyl-glycine (MAG3), which contains an N_3S, and N_2S_2 systems such as MAMA (monoamidemonoaminedithiols), DADS (N_2S diaminedithiols), CODADS and the like. These ligand systems and a variety of others are described in, for example, Liu, S. and Edwards, D., 1999. *Chem Rev.*, 99:2235-2268, and references therein.

The chelator also can include complexes containing ligand atoms that are not donated to the metal in a tetradentate array. These include the boronic acid adducts of technetium and rhenium dioximes, such as are described in US 5,183,653; US 5,387,409; and US 5,118,797, the disclosures of which are incorporated by reference herein, in their entirety.

In another embodiment, disulfide bonds of a cMet binding polypeptide of the invention are used as two ligands for chelation of a radionuclide such as ^{99m}Tc. In this way the peptide loop is expanded by the introduction of Tc (peptide-S-S-peptide changed to peptide-S-Tc-S-peptide). This also has been used in other disulfide containing peptides in the literature (Chen, J. *et al.*, 2001. *J. Nucl. Med.*, 42:1847-1855) while maintaining biological activity. The other chelating groups for Tc can be supplied by amide nitrogens of the backbone, another cystine amino acid or other modifications of amino acids.

Particularly preferred metal chelators include those of Formula 20, 21, 22, 23a, 23b, 24a, 24b and 25, set forth in FIGS. 8A-8F. Formulae 20-22 are particularly useful for lanthanides such as paramagnetic Gd^{3+} and radioactive lanthanides such as ^{177}Lu, ^{90}Y, ^{153}Sm, ^{111}In, or ^{166}Ho. Formulae 23a-24b are particularly useful for radionuclides

^{99m}Tc, ^{186}Re, or ^{188}Re. Formula 25 is particularly useful for ^{99m}Tc. These and other metal chelating groups are described in US 6,093,382 and US 5,608,110, which are incorporated by reference herein in their entirety. Additionally, the chelating group of formula 22 is described in, for example, US 6,143,274; the chelating group of formula 24 is described in, for example, US 5,627,286 and US 6,093,382, and the chelating group of formula 25 is described in, for example, US 5,662,885; US 5,780,006; and US 5,976,495.

For formulae 24a and 24b of FIG. 8E, X is either CH_2 or O; Y is C_1-C_{10} branched or unbranched alky, aryl, aryloxy, arylamino, arylaminoacyl, or arylalkyl comprising C_1-C_{10} branched or unbranched alkyl groups, hydroxy or C_1-C_{10} branched or unbranched polyhydroxyalkyl groups, C_1-C_{10} branched or unbranched hydroxy or polyalkoxyalkyl or polyhydroxy-polyalkoxyalkyl groups; J is C(=O)-, OC(=O)-, SO_2-, NC(=O)-, NC(=S)-, N(Y), NC(=NCH_3)-, NC(=NH)-, N=N-, homopolyamides or heteropolyamines derived from synthetic or naturally occurring amino acids; and n is 1-100. Other variants of these structures are described, for example, in US 6,093,382. The disclosures of each of the foregoing patents, applications and references are incorporated by reference herein, in their entirety.

The chelators can be covalently linked directly to the cMet binding moiety or multimeric polypeptide construct or linked to the cMet binding polypeptide via a linker, as described previously, and then directly labeled with the radioactive metal of choice (see, WO 98/52618, US 5,879,658, and US 5,849,261).

Complexes of radioactive technetium are particularly useful for diagnostic imaging and complexes of radioactive rhenium are particularly useful for radiotherapy. In forming a complex of radioactive technetium with the reagents of this invention, the technetium complex, preferably a salt of ^{99m}Tc pertechnetate, is reacted with the reagent in the presence of a reducing agent. Preferred reducing agents are dithionite, stannous and ferrous ions; the most preferred reducing agent is stannous chloride. Means for preparing such complexes are conveniently provided in a kit form comprising a sealed vial containing a predetermined quantity of a reagent of the invention to be labeled and a sufficient amount of reducing agent to label the reagent with ^{99m}Tc. Alternatively, the complex can be formed by reacting a peptide of this invention conjugated with an

appropriate chelator with a pre-formed labile complex of technetium and another compound known as a transfer ligand. This process is known as ligand exchange and is well known to those skilled in the art. The labile complex can be formed using such transfer ligands as tartrate, citrate, gluconate or mannitol, for example. Among the ^{99m}Tc pertechnetate salts useful with the present invention are included the alkali metal salts such as the sodium salt, or ammonium salts or lower alkyl ammonium salts.

Preparation of the complexes of the present invention where the metal is radioactive rhenium can be accomplished using rhenium starting materials in the +5 or +7 oxidation state. Examples of compounds in which rhenium is in the Re(VII) state are NH_4ReO_4 or $KReO_4$. Re(V) is available as, for example, $[ReOCl_4](NBu_4)$, $[ReOCl_4](AsPh_4)$, $ReOCl_3(PPh_3)_2$ and as $ReO_2(pyridine)^{4+}$, where Ph is phenyl and Bu is n-butyl. Other rhenium reagents capable of forming a rhenium complex also can be used.

Radioactively labeled scintigraphic imaging agents provided by the present invention are encompassed having a suitable amount of radioactivity. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected at unit dosage is from about 0.01 mL to about 10 mL. In forming ^{99m}Tc radioactive complexes, it is generally preferred to form radioactive complexes in solutions containing radioactivity at concentrations of from about 0.01 mCi to 100 mCi per mL.

Typical doses of a radionuclide-labeled cMet binding imaging agents according to the invention provide 10-20 mCi. After injection of the cMet-specific radionuclide imaging agent into the patient, a gamma camera calibrated for the gamma ray energy of the nuclide incorporated in the imaging agent is used to image areas of uptake of the agent and quantify the amount of radioactivity present in the site. Imaging of the site *in vivo* can take place in a matter of a few minutes. However, imaging can take place, if desired, in hours or even longer, after the radiolabeled peptide is injected into a patient. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos.

Proper dose schedules for the radiotherapeutic compounds of the present invention are known to those skilled in the art. The compounds can be administered

using many methods including, but not limited to, a single or multiple IV or IP injections, using a quantity of radioactivity that is sufficient to cause damage or ablation of the targeted cMet-expressing tissue, but not so much that substantive damage is caused to non-target (normal tissue). The quantity and dose required is different for different constructs, depending on the energy and half-life of the isotope used, the degree of uptake and clearance of the agent from the body and the mass of the tumor. In general, doses can range from a single dose of about 30-50 mCi to a cumulative dose of up to about 3 Ci.

The radiotherapeutic compositions of the invention can include physiologically acceptable buffers, and can require radiation stabilizers to prevent radiolytic damage to the compound prior to injection. Radiation stabilizers are known to those skilled in the art, and can include, for example, para-aminobenzoic acid, ascorbic acid, gentistic acid and the like.

A single, or multi-vial kit that contains all of the components needed to prepare the complexes of this invention, other than the radionuclide, is an integral part of this invention.

A single-vial kit preferably contains a chelating ligand, a source of stannous salt, or other pharmaceutically acceptable reducing agent, and is appropriately buffered with pharmaceutically acceptable acid or base to adjust the pH to a value of about 3 to about 9. The quantity and type of reducing agent used would depend on the nature of the exchange complex to be formed. The proper conditions are well known to those that are skilled in the art. It is preferred that the kit contents be in lyophilized form. Such a single vial kit can optionally contain labile or exchange ligands such as glucoheptonate, gluconate, mannitol, malate, citric or tartaric acid and can also contain reaction modifiers such as diethylenetriamine-pentaacetic acid (DPTA), ethylenediamine tetraacetic acid (EDTA), or α, β, or γ cyclodextrin that serve to improve the radiochemical purity and stability of the final product. The kit also can contain stabilizers, bulking agents such as mannitol, that are designed to aid in the freeze-drying process, and other additives known to those skilled in the art.

A multi-vial kit preferably contains the same general components but employs more than one vial in reconstituting the radiopharmaceutical. For example, one vial can

contain all of the ingredients that are required to form a labile Tc(V) complex on addition of pertechnetate (*e.g.*, the stannous source or other reducing agent). Pertechnetate is added to this vial, and after waiting an appropriate period of time, the contents of this vial are added to a second vial that contains the ligand, as well as buffers appropriate to adjust the pH to its optimal value. After a reaction time of about 5 to 60 minutes, the complexes of the present invention are formed. It is advantageous that the contents of both vials of this multi-vial kit be lyophilized. As above, reaction modifiers, exchange ligands, stabilizers, bulking agents, etc. can be present in either or both vials.

Therapeutic Applications

The cMet binding polypeptides and multimeric polypeptide constructs of the present invention can be used to present, treat or improve the activity of therapeutic agents such as anti-proliferative or tumoricidal agents against undesired cellular proliferation (such as occurs in neoplastic tumors, *e.g.*, cancer, by providing or improving their affinity for cMet and their residence time at a HGF/cMet complex on proliferating cells, such as, for example, epithelial cells) for diseases associated with cMet, including, but not limited to, diseases related to cMet activity. In this aspect of the invention, hybrid agents are provided by conjugating a cMet binding polypeptide or multimeric polypeptide construct according to the invention with a therapeutic agent. The therapeutic agent can be a radiotherapeutic, discussed above, a drug, chemotherapeutic or tumoricidal agent, genetic material or a gene delivery vehicle, etc. The cMet binding polypeptide moiety portion of the conjugate causes the therapeutic to "home" to the sites of cMet or HGF/cMet complex (*i.e.*, activated epithelial cells), and to improve the affinity of the conjugate for the endothelium, so that the therapeutic activity of the conjugate is more localized and concentrated at the sites of cellular proliferation. In addition, these cMet binding moieties can inhibit HGF-mediated signaling events by preventing HGF from binding to cMet. Such conjugates will be useful in treating hyperproliferative disorders, especially neoplastic tumor growth and metastasis, in mammals, including humans. The method comprises administering to a mammal in need thereof an effective amount of a cMet binding polypeptide or

multimeric polypeptide construct according to the invention conjugated with a therapeutic agent. The invention also provides the use of such conjugates in the manufacture of a medicament for the treatment of angiogenesis associated diseases in mammals, including humans.

Suitable therapeutic agents for use in this aspect of the invention include, but are not limited to: antineoplastic agents, such as platinum compounds (*e.g.*, spiroplatin, cisplatin, and carboplatin), methotrexate, adriamycin, mitomycin, ansamitocin, bleomycin, cytosine, arabinoside, arabinosyl adenine, mercaptopolylysine, vincristine, busulfan, chlorambucil, melphalan (*e.g.*, PAM, L-PAM, or phenylalanine mustard), mercaptopurine, mitotane, procarbazine hydrochloride, dactinomycin (actinomycin D), daunorubcin hydrochloride, doxorubicin hydrochloride, taxol, mitomycin, plicamycin (mithramycin), aminoglutethimide, estramustine phosphate sodium, flutamide, leuprolide acetate, megestrol acetate, tamoxifen citrate, testoiactone, trilostane, amsacrine (m-AMSA), aparaginase (L-aparaginase), *Erwina* aparaginase, etoposide (VP-16), interferon *CX*-2a, Interferon *CX*-2b, teniposide (VM-26, vinblastine sulfate (VLB), vincristine sulfate, bleomycin sulfate, adriamycin, and arabinosyl; anti-angiogenic agents such as tyrosine kinase inhibitors with activity toward signaling molecules important in angiogenesis and/or tumor growth such as SU5416 and SU6668 (Sugen/Pharmacia and Upjohn), endostatin (EntreMed), angiostatin (EntreMed), Combrestatin (Oxigene), cyclosporine, 5-fluorouracil, vinblastine, doxorubicin, paclitaxel, daunorubcin, immunotoxins; coagulation factors; antivirals such as acyclovir, amantadine azidothymidine (AZT or Zidovudine), ribavirin and vidarabine monohydrate (adenine arahinoside, ara-A); antibiotics, antimalarials, antiprotozoans such as chloroquine, hydroxychloroquine, metroidazole, quinine and meglumine antimonate; anti-inflammatories such as diflunisal, ibuprofen, indomethacin, meclofenamate, mefenamic acid, naproxen, oxyphenbutazone, phenylbutazone, piroxicam, sulindac, tolmetin, aspirin and salicylates.

In one embodiment of the invention, the therapeutic agent can be associated with an ultrasound contrast agent composition in which cMet binding moieties of the invention are linked to the material employed to form the vesicles as described herein. After administration of the ultrasound contrast agent and the optional imaging of the

contrast agent bound to the tissue expressing cMet or HGF/cMet complex, the tissue can be irradiated with an energy beam (preferably ultrasonic, *e.g.*, with a frequency of from 0.3 to 3 MHz), to rupture or burst the microvesicles. The therapeutic effect of the therapeutic agent can thus be enhanced by the energy released by the rupture of the microvesicles, in particular causing an effective delivery of the therapeutic agent to the targeted tissue. For instance, the therapeutic agent can be associated with the targeted ultrasound contrast agent and delivered as described in US 6,258,378, herein incorporated by reference.

The cMet binding polypeptides and multimeric polypeptide constructs of the present invention also can be used to target genetic material to cMet-expressing cells. Thus, they can be useful in gene therapy, particularly for treatment of hyperproliferative disorders. In this embodiment, genetic material or one or more delivery vehicles containing genetic material useful in treating a hyperproliferative disorder can be conjugated to one or more cMet binding moieties of the invention and administered to a patient. The genetic material can include nucleic acids, such as RNA or DNA, of either natural or synthetic origin, including recombinant RNA and DNA and antisense RNA and DNA. Types of genetic material that can be used include, for example, genes carried on expression vectors such as plasmids, phagemids, cosmids, yeast artificial chromosomes (YACs) and defective or "helper" viruses, antigene nucleic acids, both single and double stranded RNA and DNA and analogs thereof, such as phosphorothioate and phosphorodithioate oligodeoxynucleotides. Additionally, the genetic material can be combined, for example, with lipids, proteins or other polymers. Delivery vehicles for genetic material can include, for example, a virus particle, a retroviral or other gene therapy vector, a liposome, a complex of lipids (especially cationic lipids) and genetic material, a complex of dextran derivatives and genetic material, etc.

In a preferred embodiment the constructs of the invention are utilized in gene therapy for treatment of hyperproliferative disorders. In this embodiment, genetic material, or one or more delivery vehicles containing genetic material, *e.g.*, useful in treating a hyperproliferative disorder, can be conjugated to one or more cMet binding

polypeptides or multimeric polypeptide constructs of the invention and administered to a patient.

Constructs including genetic material and the cMet-binding moieties of the invention can be used, in particular, to selectively introduce genes into proliferating cancer cells (*e.g.*, epithelial cells), which can be useful to treat cancer.

Therapeutic agents and the cMet binding moieties of the invention can be linked or fused in known ways, optionally using the same type of linkers discussed elsewhere in this application. Preferred linkers will be substituted or unsubstituted alkyl chains, amino acid chains, polyethylene glycol chains, and other simple polymeric linkers known in the art. More preferably, if the therapeutic agent is itself a protein, for which the encoding DNA sequence is known, the therapeutic protein and cMet binding polypeptide can be coexpressed from the same synthetic gene, created using recombinant DNA techniques, as described above. The coding sequence for the cMet binding polypeptide can be fused in frame with that of the therapeutic protein, such that the peptide is expressed at the amino- or carboxy-terminus of the therapeutic protein, or at a place between the termini, if it is determined that such placement would not destroy the required biological function of either the therapeutic protein or the cMet binding polypeptide. A particular advantage of this general approach is that concatamerization of multiple, tandemly arranged cMet binding polypeptides is possible, thereby increasing the number and concentration of cMet binding sites associated with each therapeutic protein. In this manner cMet binding avidity is increased, which would be expected to improve the efficacy of the recombinant therapeutic fusion protein.

Additionally, constructs including cMet binding polypeptides of the present invention can themselves be used as therapeutics to treat a number of diseases associated with cMet activity. For example, where binding of a protein or other molecule (*e.g.*, a growth factor, hormone etc.) is necessary for or contributes to a disease process and a binding moiety inhibits such binding, constructs including such binding moieties could be useful as therapeutics. Similarly, where binding of a binding moiety itself inhibits a disease process, constructs containing such binding moieties also could be useful as therapeutics.

The binding of HGF to cMet results in the activation of numerous intracellular signal transduction pathways leading to hyperproliferation of various cells. As such, in one embodiment, constructs including cMet binding polypeptides that inhibit the binding of HGF to cMet (or otherwise inhibit activation of cMet) can be used as anti-neoplastic agents. In addition, as binding of HGF and activation of cMet is implicated in angiogenic activity, in another embodiment, constructs including cMet binding polypeptides that inhibit the binding of HGF to cMet, or otherwise inhibit activation of cMet, can be used as anti-angiogenic agents. Certain constructs of the invention including monomers, multimers and heteromultimers that inhibit activation of cMet are also discussed in the Examples, and include, for example, SEQ ID NO:365 (FIG. 10). The binding polypeptides and constructs thereof of the present invention are useful as therapeutic agents for treating conditions that involve endothelial and/or epithelial cells expressing cMet. Because an important function of endothelium is angiogenesis, or the formation of blood vessels, the polypeptides and constructs thereof are particularly useful for treating conditions that involve angiogenesis and/or hyperproliferation. Conditions that involve angiogenesis include, for example, solid tumors, tumor metastases and benign tumors. Tumors caused by cMet activation or through angiogenesis are well known in the art and include, for example, breast, thyroid, glioblastoma, prostate, malignant mesothelioma, colorectal, hepatocellular, hepatobiliary, renal, osteosarcoma and cervical. Additional tumors and related disorders are listed in Table I of U.S. Patent No. 6,025,331, issued February 15, 2000 to Moses, *et al.*, the teachings of which are incorporated herein by reference. Benign tumors include, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas. Other relevant diseases that involve angiogenesis and/or hyperproliferation include for example, rheumatoid arthritis, psoriasis, and ocular diseases, such as diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rebeosis, Osler-Webber Syndrome, myocardial angiogenesis, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma and wound granulation. Other relevant diseases or conditions that involve blood vessel growth include intestinal adhesions, atherosclerosis, scleroderma, and hypertropic scars, and ulcers. Furthermore, the

binding polypeptides and constructs thereof of the present invention can be used to reduce or prevent uterine neovascularization required for embryo implantation, for example, as a birth control agent.

The binding polypeptides, multimeric polypeptide constructs and constructs conjugates thereof can be administered to an individual over a suitable time course depending on the nature of the condition and the desired outcome. They binding polypeptides and constructs thereof can be administered prophylactically, *e.g.*, before the condition is diagnosed or to an individual predisposed to a condition. The binding polypeptides multimeric polypeptide constructs and conjugates and constructs thereof can be administered while the individual exhibits symptoms of the condition or after the symptoms have passed or otherwise been relieved (such as after removal of a tumor). In addition, they binding polypeptides and constructs thereof of the present invention can be administered a part of a maintenance regimen, for example to prevent or lessen the recurrence or the symptoms or condition. As described below, the binding polypeptides multimeric polypeptide constructs and conjugates and constructs thereof of the present invention can be administered systemically or locally.

The quantity of material administered will depend on the seriousness of the condition. For example, for treatment of a hyperproliferative disorder, *e.g.*, in the case of neoplastic tumor growth, the position and size of the tumor will affect the quantity of material to be administered. The precise dose to be employed and mode of administration must per force, in view of the nature of the complaint, be decided according to the circumstances by the physician supervising treatment. In general, dosages of the agent conjugate polypeptides, multimeric polypeptide constructs and conjugates of the present invention will follow the dosages that are routine for the therapeutic agent alone, although the improved affinity of a binding polypeptide or multimeric polypeptide construct of the invention for its target can allow for a decrease in the standard dosage.

Such conjugate pharmaceutical compositions are preferably formulated for parenteral administration, and most preferably for intravenous or intra-arterial administration. Generally, and particularly when administration is intravenous or intra-

arterial, pharmaceutical compositions can be given as a bolus, as two or more doses separated in time, or as a constant or non-linear flow infusion.

As used herein the term "therapeutic" includes at least partial alleviation of symptoms of a given condition. The binding polypeptides, multimeric constructs and constructs conjugates thereof of the present invention do not have to produce a complete alleviation of symptoms to be useful. For example, treatment of an individual can result in a decrease in the size of a tumor or diseased area, or prevention of an increase in size of the tumor or diseased area. Treatment also can prevent or lessen the number or size of metastatic outgrowths of the main tumor(s).

Symptoms that can be alleviated include physiological characteristics such as cMet activity. The binding polypeptides multimeric polypeptide constructs and conjugates and constructs thereof of the present invention can inhibit activity of cMet and its homologs by binding to cMet and inhibiting its activity or by binding to cMet and inhibiting HGF from activating this receptor. Such inhibition can be detected, for example, by measuring the phosphorylation state of the receptor in the presence of or after treatment with the binding polypeptides or constructs thereof. Based on the teachings provided herein, one of ordinary skill in the art would know how and be able to administer a suitable dose of binding polypeptide, multimeric polypeptide constructs and conjugates or construct thereof as provided herein, and measure the effect of treatment on the parameter of interest. For example, the size of the area of interest (*e.g.*, the tumor or lesion) can be measured before and after treatment. Cells or cMet itself can be isolated from the sample and used in assays described herein.

The dosage of the polypeptides multimeric polypeptide constructs and conjugates and constructs thereof can depend on the age, sex, health, and weight of the individual, as well as the nature of the condition and overall treatment regimen. The biological effects of the polypeptides multimeric polypeptide constructs and conjugates and constructs thereof are described herein. Therefore, based on the biological effects of the binding polypeptides multimeric polypeptide constructs and conjugates and constructs provided herein, and the desired outcome of treatment, the preferred dosage is determinable by one of ordinary skill in the art through routine optimization

procedures. Typically, the daily regimen is in the range of about 0.1 mg/kg to about 1 mg/kg.

The binding polypeptides moieties and constructs conjugates thereof provided herein can be administered as the sole active ingredient, optionally together with a pharmaceutically acceptable excipient, or can be administered together (*e.g.*, simultaneously or sequentially) with other binding polypeptides and constructs thereof, other therapeutic agents, or combination thereof. In addition, the binding polypeptides moieties and conjugate constructs thereof can be conjugated to therapeutic agents, for example, to improve specificity, residence time in the body, or therapeutic effect. Such other therapeutic agents include, for example, other anti-proliferative compounds, and tumoricidal compounds. The therapeutic agent also can include antibodies. Furthermore, the binding polypeptide multimeric polypeptide constructs and constructs thereof of the present invention can be used as a cancer cell homing device. Therefore, they binding polypeptide or constructs thereof can may be conjugated to nucleic acid encoding, for example, a therapeutic polypeptide, in order to target the nucleic acid to stromal cells. Once exposed to the nucleic acid conjugated binding polypeptide moiety or conjugate thereof, the stromal cells can internalize and express the conjugated nucleic acid, thereby delivering the therapeutic peptide to the target cells.

The binding polypeptides, multimeric polypeptide constructs and conjugates and constructs thereof can be administered locally or systemically by any suitable route. Suitable routes of administration include, but are not limited to, topical application, transdermal, parenteral, gastrointestinal, intravaginal, and transalveolar. Compositions for the desired route of administration can be prepared by any of the methods well known in the pharmaceutical arts, for example, as described in Remington: *The Science and Practice of Pharmacy*, 20th ed., Lippincott, Williams and Wilkins, 2000.

For topical application, the binding polypeptides, multimeric polypeptide constructs and conjugates thereof can be suspended, for example, in a cream, gel or rinse that allows the polypeptides or constructs to penetrate the skin and enter the blood stream, for systemic delivery, or contact the area of interest, for localized delivery. Compositions suitable for topical application include any pharmaceutically acceptable base in which the polypeptides or constructs are at least minimally soluble.

For transdermal administration, the polypeptides, multimeric polypeptide constructs and conjugates thereof can be applied in pharmaceutically acceptable suspension together with a suitable transdermal device or "patch". Examples of suitable transdermal devices for administration of the polypeptides or constructs of the present invention are described, for example, in U.S. Patent No. 6,165,458, issued December 26, 2000 to Foldvari et al., and U.S. Patent No. 6,274,166B1, issued August 4, 2001 to Sintov *et al.*, the teachings of which are incorporated herein by reference.

For parenteral administration, the polypeptides, multimeric polypeptide constructs and conjugates thereof can be injected intravenously, intramuscularly, intraperitoneally, or subcutaneously. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Other pharmaceutically acceptable carriers include, but are not limited to, sterile water, saline solution, and buffered saline (including buffers like phosphate or acetate), alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, paraffin, etc. Where necessary, the composition also can include a solubilizing agent and a local anaesthetic such as lidocaine to ease pain at the site of the injection, preservatives, stabilizers, wetting agents, emulsifiers, salts, lubricants, etc. as long as they do not react deleteriously with the active compounds. Similarly, the composition may comprise conventional excipients, *i.e.* pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active compounds. Generally, the ingredients will be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent in activity units. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade "water for injection" or saline. Where the composition is to be administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

For gastrointestinal and intravaginal administration, the polypeptides, multimeric polypeptide constructs and conjugates thereof can be incorporated into

pharmaceutically acceptable powders, pills or liquids, and suppositories for rectal or vaginal administration.

For transalveolar, buccal or pulmonary administration, the polypeptides, multimeric polypeptide constructs and conjugates thereof can be suspended in a pharmaceutically acceptable excipient suitable for aerosolization and inhalation or as a mouthwash. Devices suitable for transalveolar administration such as atomizers and vaporizers also are included within the scope of the invention. Suitable formulations for aerosol delivery of polypeptides, etc. using buccal or pulmonary routes can be found, for example in U.S. Patent No. 6,312,665B1, issued November 6, 2001 to Pankaj Modi, the teachings of which are incorporated herein by reference.

In addition, the polypeptides, multimeric polypeptide constructs and conjugates thereof of the present invention can be administered nasally or ocularly, where the polypeptide or construct is suspended in a liquid pharmaceutically acceptable agent suitable for drop-wise dosing.

The polypeptides, multimeric polypeptide constructs and conjugates thereof of the present invention can be administered such that the polypeptide, etc. is released in the individual over an extended period of time (sustained or controlled release). For example, the polypeptide, multimeric polypeptide constructs and conjugates thereof can be formulated into a composition such that a single administration provides delivery of the polypeptide, etc. for at least one week, or over the period of a year or more. Controlled release systems include monolithic or reservoir-type microcapsules, depot implants, osmotic pumps, vesicles, micelles, liposomes, transdermal patches and iontophoretic devices. In one embodiment, the polypeptides, multimeric polypeptide constructs and conjugates thereof of the present invention are encapsulated or admixed in a slowly degrading, non-toxic polymer. Additional formulations suitable for controlled release of the polypeptides, multimeric polypeptide constructs and conjugates thereof provided herein are described in U.S. Patent No. 4,391,797, issued July 5, 1983, to Folkman *et al.*, the teachings of which are incorporated herein by reference.

Another suitable method for delivering the polypeptides of the present to an individual is via *in vivo* production of the polypeptide. A gene encoding the polypeptide can be administered to the individual such that the encoded polypeptide is

expressed. The gene can be transiently expressed. In a particular embodiment, the gene encoding the polypeptide is transfected into cells that have been obtained from the patient, a method referred to as *ex vivo* gene therapy. Cells expressing the polypeptide are then returned to the patient's body. Methods of *ex vivo* gene therapy are well known in the art and are described, for example, in U.S. Patent No. 4,391,797, issued March 21, 1998 to Anderson *et al.*, the teachings of which are incorporated herein by reference.

Isolation of cMet binding moieties polypeptides and preparation and use of cMet binding moieties and conjugates thereof in accordance with this invention will be further illustrated in the following examples. The specific parameters included in the following examples are intended to illustrate the practice of the invention, and they are not presented to in any way limit the scope of the invention.

Examples

Example 1: Method for identification of cMet-binding polypeptides

A four-pronged selection strategy using a variety of peptide-displaying phage libraries was utilized to screen for cMet-binding polypeptides. Both the extracellular domain of the cMet receptor (expressed as an Fc-fusion protein) and the colorectal cancer cell line, DLD-1, which express high levels of cMet on their cell surface, were used as tools for the selections.

Briefly, the selections involved either using the soluble cMet-Fc-fusion protein or DLD-1 cells as the target. Specific elutions with HGF (first for 1 hour and then overnight to identify both low and high affinity cMet binders) were performed. Additionally, while using the soluble cMet receptor, all peptide-displaying phage that remained bound to the receptor were harvested to identify peptides that did not bind to the ligand binding site, but could nevertheless be potentially developed into imaging agents. FIG. 9 illustrates the selection strategy that was employed. Briefly, 21 different selection campaign/elution combinations were performed with each library pool. An additional 10 selection campaigns representing rounds 3 and 4 using the soluble Met-Fc

fusion protein were also performed. HGF elutions were at a concentration of 100 ng/mL.

Example 2: Determination of peptide-displaying phage binding to soluble cMet-Fc fusion protein "protein phage ELISAs"

Protein phage ELISAs using peptide-displaying phage isolates from the various selection campaigns were performed to determine specificity of the peptides for cMet versus an unrelated Fc-fusion protein (TRAIL-Fc). Briefly, 384-well plates were coated overnight at 4C with 0.5 μg/mL of cMet-Fc fusion protein or TRAIL-Fc fusion protein (background). The plates were blocked for 2 hours 37C with 3% (w/v) BSA in PBS containing 0.05% (v/v) Tween-20 (PBST). The plates were washed with PBST and 100 μL of peptide-displaying phage were added to each well. The plates were incubated for 2 hours at room temperature and washed with PBST. cMet-binding peptide-displaying phage were detected using an HRP-conjugated anti-M13 antibody.

The peptide-displaying phage that demonstrated a > 3-fold binding to cMet-Fc fusion protein versus TRAIL-Fc fusion protein are herein referred to as "positive hits". The positive hits identified in the above screen were subjected to DNA sequencing. From subsequent sequence analysis, 187 unique peptide sequences were identified. The corresponding amino acid sequences of the cMet-binding phage-displayed peptides are listed in Table 1 (SEQ ID NO: 001-101, 365-387, 390-404, 449-496).

Example 3: Determination of cMet binding in a cellular model

Whole cell ELISAs were performed to assess whether the positive hits demonstrated specific binding to cell surface-expressed human cMet.

Whole cell ELISAs were performed using 3T3 cells that over-express human cMet. 3T3 cells that do not express cMet ("non-expressing cells") were used as a control cell line. Briefly, 96-well plates were seeded with 10^5 cells per well. The plates were centrifuged for 5 minutes at 1600 rpm to pellet the cells. The resulting cell layer was fixed with 0.1% (v/v) glutaraldehyde for 12 minutes at 37C. The cells were washed with PBS and subsequently blocked with 3% BSA in PBST for 1 hour at 37C. Peptide-displaying phage also were blocked in the above solution for 1 hour at 37C.

100 µL of blocked phage was then added to each well and the plates were incubated for 1 hour at room temperature. The plates were washed with PBST. cMet-binding peptide-displaying phage were detected using an HRP-conjugated anti-M13 antibody.

Example 4: HGF competition protein ELISAs

HGF competition protein ELISAs were performed in an attempt to determine whether any of the cMet-binding peptides compete with HGF for a similar binding site on cMet. This competition ELISA identifies peptides that serve as "HGF antagonistic peptides", peptides that block HGF-mediated signaling events (*e.g.*, proliferation). These assays were conducted using the peptide-displaying phage discovered from the initial selection and screening campaigns using the first generation peptide libraries. Briefly, 96-well plates were coated overnight at 4C with 0.5 µg/mL of cMet-Fc fusion protein or TRAIL-Fc fusion protein (background). The plates were blocked for 2 hours at 37C with 3% BSA in PBST. The plates were washed with PBST, and 100 µL of HGF (either at 100 ng/mL or 500 ng/mL in PBST) was added to each well. The plates were incubated for 30 minutes at room temperature after which the plates were washed with PBST and 70 µL of HGF (143 ng/mL or 714 ng/mL) or 70 µL of PBST was added to the respective wells. This was followed by an addition of 30 µL of peptide-displaying phage overnight culture to each well. The plates were incubated for 2 hours at room temperature, washed with PBST and cMet-binding peptide-displaying phage was detected using an HRP-conjugated anti-M13 antibody.

Data for the protein ELISAs, whole cell ELISAs and the HGF competition experiments is presented in Table 7.

Example 5: Peptide synthesis and fluorescein labeling

A select number of cMet-binding peptides corresponding to positive phage isolates were synthesized on a solid phase matrix using 9-fluorenylmethoxycarbonyl protocols. These peptides were purified with reverse phase chromatography. Peptide masses were confirmed by electrospray mass spectrometry, and peptides were quantified by measuring absorbance at 280 nm. For synthesis, two N-terminal and two C-terminal amino acids from the phage vector sequence from which the peptide was

excised were retained, and a linker, *e.g.*, -Gly-Gly-Gly-Lys-NH_2 (SEQ ID NO:513) was added to the C-terminus of each peptide. Each peptide was N-terminally acetylated. Selected lysine residues were protected with 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) where appropriate. The protecting group allows for selective coupling to the C-terminal lysine, is not removed during peptide cleavage, but can be removed after coupling with 2% hydrazine in DMF or 0.5 M hydroxylamine, pH 8, in water.

Each peptide was labeled with fluorescein on the C-terminal lysine using fluorescein (N-hydroxysuccinimide ester derivative) or fluorescein isothiocyanate (FITC) in DMF with 2% diisopropylethylamine (DIPEA). In the case where the peptide contained an ivDde protected lysine, the reaction was quenched by the addition of 2% hydrazine, which reacts with all free NHS-fluorescein and removes the internal protecting group. For all other peptides, the reaction was quenched by the addition of an equal volume of 0.5M hydroxylamine, pH 8. The quenched reactions were then diluted with water to less than 10% DMF and then purified using C18 reverse phase chromatography. The peptides were verified by analyzing them for expected mass using an LC-MS system (HP1100 HPLC with in-line SCIEX AP150 single quadrapole mass spectrometer), and the purity of the peptides was determined.

Example 6: Fluorescence anisotropy measurements

Fluorescence anisotropy measurements were performed in 384-well microplates in a volume of 10 μL in binding buffer (PBS, 0.01% Tween-20, pH 7.5) using a Tecan Polarion fluorescence polarization plate reader (Caracas, Venezuela). The concentration of fluorescein-labeled peptide was held constant (20 nM) and the concentration of cMet-Fc fusion protein (or similar target) was varied. Binding mixtures were equilibrated for 10 minutes in the microplate at 30C before measurement. The observed change in anisotropy was fit to the equation below via nonlinear regression to obtain the apparent K_D. This equation (1) assumes that the synthetic peptide and cMet form a reversible complex in solution with 1:1 stoichiometry.

$$r_{obs} = r_{free} + (r_{bound} - r_{free}) \frac{(K_D + cMet + P) - \sqrt{(K_D + cMet + P)2 - 4 \cdot cMet \cdot P}}{2 \cdot P}$$

where r_{obs} is the observed anisotropy, r_{free} is the anisotropy of the free peptide, r_{bound} is the anisotropy of the bound peptide, K_D is the apparent dissociation constant, cMet is the total cMet concentration, and P is the total fluorescein-labeled peptide concentration. K_D was calculated in a direct binding assay ($K_{D,B}$) and therefore these values represent cMet binding to the fluorescein labeled peptide.

Example 7: Peptide competition fluorescence polarization assays

Peptide competition fluorescence polarization assays were performed to determine which peptides compete with each other for binding to cMet. This would identify potential heteromeric peptide complexes that exhibit higher affinity for the cMet receptor than an individual peptide alone.

Briefly, cross competition of cMet-binding peptides was performed on a Cartesian liquid handler (Irvine, CA) in a 3 μL total reaction volume. Flourescein-labeled peptides were diluted to a final concentration of 20 nM and unlabeled competitor peptides were diluted to a final concentration of 10 μM. cMet-Fc fusion protein was diluted to the K_D for each fluorescein-labeled peptide in the reaction. Binding mixtures were equilibrated for 10 minutes on the microplate at 30C before measuring any changes in anisotropy. From these studies, three pairs of cMet-binding peptides were identified as being non-competitors and represent ideal candidates for heteromeric cMet-binding peptide complexes (see Table 9).

Example 8: General procedure for preparation of heteromeric cMet-binding peptide complexes

Each of the dimers consists of a Tc-chelating 6-PnAO ligand bearing sequence (generally referred to as A) and a spacer functionalized (spacer = JJ; J = 8-Amino-3,6-dioxaoctanoic acid) portion (generically referred to as B). Compound B was treated

with a 10-fold excess of glutaric acid bis NHS ester (Tyger Scientific, Princeton, NJ) and ~20-fold excess of diisopropylethylamine at ambient temperature in DMF for 30 minutes. The reaction mixture was diluted with ether (15-fold by volume) which led to the precipitation of the mono-NHS ester of the glutarylated peptide. The ether was decanted and the solid washed thrice more with ether, which removed any traces of unreacted glutaric acid bis NHS ester. The resulting solid was resuspended in dry DMF and the compound A (1 equiv) was added followed by diisopropylethylamine (20 equiv) and the mixture was stirred for 24 hours at ambient temperature. The mixture was diluted with water (50-fold) and the mixture was directly loaded onto a reverse-phase HPLC column, which was eluted with a gradient of acetonitrile (0.1% TFA) into water (0.1% TFA). Fractions containing the desired product were combined and lyophilized to provide the desired materials.

Specific Example: Preparation of Heterodimeric cMet-binding Peptides Complexes

1) Preparation of a PnAOG-Glut modified SEQ ID NO:514 peptide (a type A compound)

To a solution of 6-Glutaryl-PnAO (40 mg, 0.1 mmol) in dry DMF (0.2 mL) was added N-hydroxysuccinimide (NHS, 14 mg, 0.12 mmol) and diisopropylcarbodiimide (DIC, 15 mg, 0.12 mmol) and stirred for 4 h at room temperature. Ether:hexane (5 mL, 1:1) was added to the reaction mixture. The mixture was stirred and the supernatant solution was removed by decantation, leaving behind the paste in the flask. The paste was washed with ether:hexane (1:1) (3 × 5 mL) and dissolved in dry DMF (0.2 mL). To this solution were added the K-(ivDde)-modified SEQ ID NO:518 (50 mg, 0.017 mmol) and diisopropylethylamine (DIEA, 10 mg, 0.08 mmol) and the resultant mixture was stirred for 18 hours. Hydrazine (10 μL) was added and the solution was stirred for 30 min. The reaction mixture was diluted with water (20 mL), loaded onto a reversed-phase (C18) HPLC column, and eluted with water (0.1% TFA)-acetonitrile (0.1% TFA) system. Fractions containing the required product (>95% purity) were collected and freeze-dried to provide SEQ ID NO:518-(6-PnAO-Glut)) (see Scheme 5 as shown in FIG. 11) as a colorless fluffy solid. The yield was 25.1 mg (47.4%).

2) Preparation of Dimer containing SEQ ID NO:514 linked to SEQ ID NO:515

To a solution of the peptide containing SEQ ID NO:515 (a type B compound) (10 mg, 0.0034 mmol) and diisopropylethylamine (10 mg, 0.08 mmol) in dry DMF (0.2 mL) was added disuccinimidyl glutarate (10 mg, 0.031 mmol) and stirred at room temperature for 30 min. The reaction mixture was diluted with ether (3 mL) and stirred. The supernatant was decanted, leaving behind the semi-solid in the flask. This process of washing the reaction product was repeated with ether (3 × 5 mL). The semi-solid thus obtained was dissolved in dry DMF (0.2 mL) and the peptide SEQ ID NO:514-(6-PnAO-Glut)) (10 mg, 0.0032 mmol) and diisopropylethylamine (10 mg, 0.08 mmol) were added and stirred the reaction mixture for 24 h at room temperature. The reaction mixture was diluted with water (10 mL), loaded onto a reversed-phase (C18) HPLC column, and eluted with water (0.1% TFA)-acetonitrile (0.1% TFA) system. Fractions containing the required product (>95% purity) were collected and freeze-dried to provide the heterodimer having SEQ ID NO:514 linked to SEQ ID NO:515 via a 6-PnAO-Glut linkage (see Scheme 6 as shown in FIG. 12) as a colorless fluffy solid. Yield: 6.7 mg (33%). The structures for this and other heterodimers are shown in FIGS. 13A-13C.

Example 9: Cellular proliferation assay

Cellular proliferation assays were performed to identify cMet-binding peptides that antagonize HGF-stimulated proliferation. These *in vitro* studies utilized a leomyosarcoma cell line, SK-LMS-1, in which cells proliferate in response to HGF. SK-LMS-1 cells were seeded on 96-well plates at a density of 2000 cells/well. After a 24 hour incubation at 37C, the cells were starved in culture media containing 0.1%BSA instead of 10% fetal bovine serum for 36 hours at 37C. Fresh starvation media with or without a cMet-binding peptide (10 μM) was added to the respective wells and the cells were incubated for 2 hours at 37C. DMF was used as the control vehicle and did not receive a cMet-binding peptide. HGF was then added at a concentration of either 50 ng/mL or 100 ng/mL and the cells were incubated for an additional 12 hours at 37C. Proliferation was assessed by measuring the incorporation of BrdU (Calbiochem, San

Diego, CA) as described by the manufacturer. Results are shown for SEQ ID NO:365 (FIG. 10).

Example 10: Design of a second generation cMet-binding peptide library

Initial selection from linear and cyclic peptide libraries identified a number of positive hits for cMet. The TN9 hits contained a highly conserved motif (CxGpPxFxC, SEQ ID NO:512, the 'p' is less strongly selected than are the uppercase amino acids). A library was constructed having both cyclic and linear members and was built in phage having a gene III stump display.

Table 1: TN9 and linear components in the second generation library:

Libraries of TN9s for cMet (cMet TN9 2nd lib #1)

E = 0.64A + 0.12C + 0.12G + 0.12T
Q = 0.12A + 0.64C + 0.12G + 0.12T
J = 0.12A + 0.12C + 0.64G + 0.12T
Z = 0.12A + 0.12C + 0.12G + 0.64T
Note: $(0.64)^{36} = 1.E-7$
$(0.64)^{39} = 2.5 E-8$

Component 1: TN9 consensus with 3 AA left extension

```
                        S   M   G       S   E   T   R   P   T
    ctcagcagtcactgtct tCC ATG Ggt     tct gaa act cgc cct aca
                        NcoI.....

 e   a   g   s   w   h   C   s   G   P   P   t   F   e   C   w   w   y
jej jqz jjz ejz zjj qez tgt ejz ggt cct cct eqj ttc jej tgc zjj zjj zez

    G   T   E   P   T   E   A   S
   gga acg gag ccg act gaa GCT AGC Gtga ctctgacagtctctgt
```

*Nhe*I...

(SEQ ID NO:518)

Met TN9 2nd lib #2: TN9 consensus with 3 AA right extension.

```
                    S   M   G       S   E   T   R   P   T
 ctcagcagtcactgtct tcc atg ggt     tct gAa act cgc cct AcA
                   NcoI.....

E   A   G   s   w   h   C   s   G   P   P   t   F   e   C   w   w   y
AG GCT GGT ejz zjj qez tgt ejz ggt cct cct eqj ttc jej tgc zjj zjj zez

    g   t   e   P   T   E   R   P   S   S   S
   jjz eqj jej ccg AcT gAA cgt cct agt GCT AGC Gtga ctctgacagtctctgt
                                        NheI...
```

SEQ ID NO:519)

Met TN9 2nd lib #3 SIQCKGPPWFSCAMY (SEQ ID NO:537) with 3 AA extension on left

```
                    S   M   G       S   E   T   R   P   T
 ctcagcagtcactgtct tcc atg ggt     tct gaa act cgc cct AcA
                   NcoI.....

e   a   g   s   i   q   C   k   G   P   P   w   F   s   C   a   m   y
ej jqz jjz ejz ezz qej tgc eej ggt cct cct zjj ttc ejz tgt jqj ezj zez

    G   T   E   P   T   E   A   S   A
   ggA Acg gAg ccg AcT gAA GCT AGC Gtga ctctgacagtctctgt
```

cMet TN9 2nd lib #4 SIQCKGPPWFSCAMY (SEQ ID NO:537) with 3 AA extension on right

```
                    S   M   G       S   E   T   R   P   T
  ctcagcagtcactgtct tcc atg ggt     tct gaa act cgc cct AcA
                    NcoI.....

 E   A   G   s   i   q   C   k   G   P   P   w   F   s   C   a   m   y
gag gcc ggt ejz ezz qej tgc eej ggt cct cct zjj ttc ejz tgt jqj ezj zez

     g   t   e   P   T   E   R   P   S   S   A
    jjz eqj jej ccg AcT gAA cgt cct agt GCT AGC Gtga ctctgacagtctctgt
                                      NheI...
```

(SEQ ID NO:521)

cMet TN9 5th lib 330-F05 YYGCKGPPTFECQWM (SEQ ID NO:531)with 3 AA extension on right

three peptides have the core sequence CKGPPTFEC (SEQ ID NO:653)

```
                    S   M   G       S   E   T   R   P   T
  ctcagcagtcactgtct tcc atg ggt     tct gAa act cgc cct AcA
                    NcoI.....

 E   A   G   y   y   g   C   k   G   P   P   t   F   e   C   q   w   m
GAG GCT GGT zez zez jjz tgc eej ggt cct cct eqz ttc jej tgt qee zjj ezj

     g   t   e   P   T   E   R   P   S   S   S
    jjz eqj jej ccg AcT gAA cgt cct agt GCT AGC Gtga ctctgacagtctctgt
                                      NheI...
```

(SEQ ID NO:522)

cMet TN9 6th lib: 550-G12 AFFCSGPPTFMCSLY (SEQ ID NO:536) with 3 AA extension on right

two peptides have the core sequence CSGPPTFMC (SEQ ID NO:654)

```
                    S  M  G      S  E  T  R  P  T
ctcagcagtcactgtct tcc atg ggt    tct gAa act cgc cct AcA
                   NcoI.....

E  A  G  a  f  f  C  s  G  p  P  t  F  m  C  s  l  y
GAG GCT GGT jqz zzq zzq tgt zqz ggt qqj cct eqz ttc ezj tgc ejq qzz zez

   g  t  e  P  T  E  R  P  S  S  S
jjz eqj jej ccg AcT gAA cgt cct agt GCT AGC Gtga ctctgacagtctctgt
                                   NheI...
```

(SEQ ID NO:523)

cMet TN9 7th lib, three AA to left and let first P of gPP vary.

```
                    S  M  G      S  E  T  R  P  T
ctcagcagtcactgtct tCC ATG Ggt    tct gaa act cgc cct aca
                   NcoI.....

e  a  g  q  f  k  C  a  G  p  P  s  F  a  C  w  m  t
jej jqz jjz qej zzq eej tgt jqz ggt qqj ccg ejz ttc jqq tgt zjj ezj eqq

   G  T  E  P  T  E  A  S
gga acg gag ccg act gaa GCT AGC Gtga ctctgacagtctctgt
                       NheI...
```

(SEQ ID NO:524)

Example 11: Analysis of 94-E08 and other linear peptides selected for binding cMet.

The linear isolate 94-E08 (SEQ ID NO:454) has high affinity for cMet yet there were few other peptides isolated that had any homology to 94-E08 and those that did have very limited similarity over very short regions. Thus, three variable oligonucleotides based on 94-E08 were made: (1) vary the first 13 codons, keeping the

last 7 constant; (2) vary 13 of the first 18, keeping 5 that showed some similarity to other isolates fixed; and (3) vary the last 13 codons, keeping the first 5 fixed, see table 4 below.

Table 4.

Component #8 with variation in the first 13 positions (SEQ ID NO:594).

```
                S   M   G     S   E
5'-tcactgtct tCC ATG Ggt   tct gaa-
   Scab.......| NcoI |

   y   d   t   w   v   f   q   f   i   h
  zez jez eqz zjj jzj zzz qej zzz ezz qez -

   e   v   p   G   E   L   V   A   M   Q
  jej jzj qqj ggt gag ctg gtt gct atg cag -

   G   G   S   G   T   E   A   S
  ggt ggt agt ggt act gaa GCT AGC Gtga ctctgac-3'
                        | NheI  |Scab........
```

Component#9 Fix five AAs and extend variegation to position 18 (SEQ ID NO:595).

```
                S   M   G     S   E
5'-tcactgtct tCC ATG Ggt   tct gaa-
   Scab.......| NcoI |

   y   D   T   w   v   F   q   f   i   h
  zez gat act zjj jzj ttt qej zzz ezz qez -

   E   V   p   g   e   l   v   a   M   Q
  gag gtt qqj jjz jej qzj jzj jqj atg caa!
```

```
      G   G   S   G   T   E   A   S
   ggt ggt agt ggt act gaa GCT AGC Gtga ctctgac-3'
                          | NheI  |Scab.......
```

Component#10 Fix first seven AAs and vary last 13 (SEQ ID NO:596).

```
                S   M   G     S   E
   5'-tcactgtct tCC ATG Ggt   tct gaa-
     Scab.......| NcoI |

      Y   D   T   W   V   F   Q   F   i   h
     tat gat act tgg gtt ttt caa ttt ezz qez -

      e   v   p   g   e   l   v   a   m   q
     jej jzz qqj jjz jej qzj jzj jqj ezj qzz!

      G   G   S   G   T   E   A   S
     ggt ggt agt ggt act gaa GCT AGC Gtga ctctgac-3'
                          | NheI  |Scab........
```

Oligonucleotide design for construction of the second generation peptide library (SEQ ID NOS:597-646; N.B. oligonucleotides marked "[RC]" consist of the reverse complement of the sequence shown):

```
  vg#1                                    NcoI....
(CM2_ZTPSAlt)    5'-        tcactgtct tcc atg ggt tct gAa-
3'
(CM2_TPLalt)     5'-        tcactgtct tcc atg ggt tct gAa
act cgc cct AcA-3'
(CM2_ZTPS)       5'-ctcagcagtcactgtct tcc at-3'
```

(CM2_V1) 5'-tct gAa
act cgc cct AcA -
jej jqz jjz ejz zjj qez tgt ejz ggt cct cct eqj ttc jej
tgc zjj zjj zez -
gga acg gag ccg act gaa gct-3'
(CM2_BPL1) [RC] 5'- gga acg gag ccg act gaa GCT AGC Gtga
ctctgacagtctctgt-3'
(CM2_XBPS) [RC] 5'-CA Gtga
ctctgacagtctctgt-3'
(BPL1_CM2) [RC] 5'- gga acg gag ccg act gaa GCT AGC Gtga
ctctgac -3'
(XBPS_CM2) [RC] 5'-act gaa GCT AGC Gtga
ctctgac -3'
*Nhe*I...

vg#2
(CM2_ZTPS) 5'-ctcagcagtcactgtct tcc at-3'
(CM2_TPLong) 5'-ctcagcagtcactgtct tcc atg ggt tct gAa
act cgc cct AcA-3'
(CM2_V2) 5'-tct gAa
act cgc cct AcA -
GAG GCT GGT ejz zjj qez tgt ejz ggt cct cct eqj ttc jej
tgc zjj zjj zez -
jjz eqj jej ccg AcT gAA cgt cct agt g-3'
(CM2_2BPL) [RC] 5'- ccg AcT gAA cgt cct agt GCT AGC Gtga
ctctgacagtctctgt-3'
(CM2_XBPS) [RC] 5'-CA Gtga
ctctgacagtctctgt-3'
(BPL2_CM2) [RC] 5'- ccg AcT gAA cgt cct agt GCT AGC Gtga
ctctgac -3'
(XPL2_CM2) [RC] 5'- ct agt GCT AGC Gtga
ctctgac -3'

(CM2_ZTPS) 5'-ctcagcagtcactgtct tcc at-3'
(CM2_TPLong) 5'-ctcagcagtcactgtct tcc atg ggt tct gAa act cgc cct AcA-3'
(CM2_V3) 5'-tct gaa act cgc cct AcA -
jej jqz jjz ejz ezz qej tgc eej ggt cct cct zjj ttc ejz tgt jqj ezj zez -
ggA Acg gAg ccg AcT gAA GC-3'
(CM2_BPL1) [RC] 5'- gga acg gag ccg act gaa GCT AGC Gtga ctctgacagtctctgt-3'
(CM2_XBPS) [RC] 5'-CA Gtga ctctgacagtctctgt-3'

vg#4
(CM2_ZTPS) 5'-ctcagcagtcactgtct tcc at-3'
(CM2_TPLong) 5'-ctcagcagtcactgtct tcc atg ggt tct gAa act cgc cct AcA-3'
(CM2_V4) 5'-tct gaa act cgc cct AcA -
gag gcc ggt ejz ezz qej tgc eej ggt cct cct zjj ttc ejz tgt jqj ezj zez -
jjz eqj jej ccg AcT gAA cgt cct agt GC -3'
(CM2_2BPL) [RC] 5'- ccg AcT gAA cgt cct agt GCT AGC Gtga ctctgacagtctctgt-3'
(CM2_XBPS) [RC] 5'-CA Gtga ctctgacagtctctgt-3'

vg#5
(CM2_ZTPS) 5'-ctcagcagtcactgtct tcc at-3'
(CM2_TPLong) 5'-ctcagcagtcactgtct tcc atg ggt tct gAa act cgc cct AcA-3'

(CM2_V5) 5'-tct gAa
act cgc cct AcA -
GAG GCT GGT zez zez jjz tgc eej ggt cct cct eqz ttc jej
tgt qee zjj ezj -
jjz eqj jej ccg AcT gAA cgt cct agt GC-3'
(CM2_2BPL) [RC] 5'- ccg AcT gAA cgt cct agt GCT AGC Gtga
ctctgacagtctctgt-3'
(CM2_XBPS) [RC] 5'-CA Gtga
ctctgacagtctctgt-3'

vg#6

(CM2_ZTPS) 5'-ctcagcagtcactgtct tcc at-3'
(CM2_TPLong) 5'-ctcagcagtcactgtct tcc atg ggt tct gAa
act cgc cct AcA-3'
(CM2_V6) 5'-tct gAa
act cgc cct AcA -
GAG GCT GGT jqz zzq zzq tgt zqz ggt qqj cct eqz ttc ezj
tgc ejq qzz zez
jjz eqj jej ccg AcT gAA cgt cct agt GC-3'
(CM2_2BPL) [RC] 5'- ccg AcT gAA cgt cct agt GCT AGC Gtga
ctctgacagtctctgt-3'
(CM2_XBPS) [RC] 5'-CA Gtga
ctctgacagtctctgt-3'

vg#7

(CM2_ZTPS) 5'-ctcagcagtcactgtct tcc at-3'
(CM2_TPLong) 5'-ctcagcagtcactgtct tcc atg ggt tct gAa
act cgc cct AcA-3'
(CM2_V7) 5'-tct gaa
act cgc cct aca -
jej jqz jjz qej zzq eej tgt jqz ggt qqj ccg ejz zzq jqq
tgt zjj ezj eqq -
gga acg gag ccg act gaa GC-3'

(CM2_BPLT) [RC] 5'-gga acg gag ccg act gaa GCT AGC Gtga ctctgacagtctctgt-3'

(CM2_XBPS) [RC] 5'-CA Gtga ctctgacagtctctgt-3'

Component#8 Vary the first 13 positions.

(CM2_ZTPSAlt) 5'-tcactgtct tcc atg ggt tct gAa-3'
(CM2C8vg) 5'-tcactgtct tCC ATG Ggt tct gaa-
zez jez eqz zjj jzj zzz qej zzz ezz qez -
jej jzj qqj ggt gag ctg gtt gct atg cag -
ggt ggt agt ggt act gaa **GCT**
(L20botamp) [RC] 5'-ggt ggt agt ggt act gaa **GCT** AGC Gtga ctct-3'

Component#9 Fix five AAs and extend variegation to position 18.

(CM2_ZTPSAlt) 5'-tcactgtct tcc atg ggt tct gAa-3'
(CM2C9vg) 5'-tcactgtct tCC ATG Ggt tct gaa-
zez gat act zjj jzj ttt qej zzz ezz qez -
gag gtt qqj jjz jej qzj jzj jqj atg caa-
ggt ggt agt ggt act gaa **GCT**-3'
(L20botamp) [RC] 5'-ggt ggt agt ggt act gaa **GCT** AGC Gtga ctct-3'

Component#10 Fix first seven AAs and vary last 13.

(CM2_ZTPSAlt) 5'-tcactgtct tcc atg ggt tct gAa-3'
(CM2C10vg) 5'-tcactgtct tCC ATG Ggt tct gaa-
tat gat act tgg gtt ttt caa ttt ezz qez -
jej jzz qqj jjz jej qzj jzj jqj ezj qzz-
ggt ggt agt ggt act gaa **GCT**-3'

(L20botamp) [RC] 5'-ggt ggt agt ggt act gaa GCT AGC Gtga ctct-3'

Example 12: Construction of a second generation cMet-binding peptide library

The phage vector DY3P82 was digested with *Nhe*I and *Nco*I, cleaned and treated with alkaline phosphatase. The 10 templates, CM2-V1 through CM2-V7, plus CM2-V8vg, CM2-V9vg and CM2-V10vg, were amplified separately, using the primer pairs listed in Table 5 below.

Table 5.

Template	Sense	Antisense
CM2_V1	CM2_TPLONG	CM2_BPL1
CM2_V2	CM2_TPLONG	CM2_BPL1
CM2_V3	CM2_TPLONG	CM2_BPL1
CM2_V4	CM2_TPLONG	CM2_BPL1
CM2_V5	CM2_TPLONG	CM2_BPL1
CM2_V6	CM2_TPLONG	CM2_BPL1
CM2_V7	CM2_TPLONG	CM2_BPL1
CM2_V8vg	CM2_ZTPSALT	L20BOTAMP
CM2_V9vg	CM2_ZTPSALT	L20BOTAMP
CM2_V10vg	CM2_ZTPSALT	L20BOTAMP

Each sample was digested separately with *Nhe*I and *Nco*I, extracted with phenol/chloroform, and mixed in an equimolar ratio prior to performing the ligation. A vector:insert ratio of 1:5 was used. Ligated DNA constructs were electroporated into DH5α cells. The resulting library size was 1.12×10^8 different transformants.

Example 13: Measurement of binding of peptide dimers to cMet

Using a BIAcore machine, the binding constants were determined for the peptide dimers (shown in FIGS. 13A-13C) binding to immobilized cMet-Fc.

Three densities of cMet-Fc (R&D Systems) were cross- linked to the dextran surface of a CM5 sensor chip by the standard amine coupling procedure (3 μM solution

diluted 1:100, 1:50, or 1:20 with 50 mM acetate, pH 5.5). Flow cell 1 was activated and then blocked to serve as a reference subtraction.

Final immobilization levels achieved:

R_L Fc 2 cMet-Fc = 2582

R_L Fc 3 cMet-Fc = 5048

R_L Fc 4 cMet-Fc = 9721

Experiments were performed in PBST buffer (5.5 mM phosphate, pH 7.65, 0.15 M NaCl) + 0.05% (v/v) Tween-20). Peptide dimers were dissolved in deionized H_2O to 1 mg/mL solutions. Dimers were diluted to 50 nM in PBS. Serial dilutions were performed to produce 25, 12.5, 6.25, and 3.125 nM solutions. All samples were injected in duplicate. For association, dimers were injected at 30 µL/minute for 3 minutes using the kinject program. Following a 10-minute dissociation, any remaining peptide was stripped from the cMet surface with two quickinjects of 4M $MgCl_2$ for 2 minutes at 50 µL/minute. Sensorgrams were analyzed using BIAevaluation software 3.1. The heterodimer, Ac-GSPEMCMMFPFLYPCNHHAPGGGK{PnAO6-Glut-K[Ac-GSFFPCWRIDRFGYCHANAPGGGKJJ-Glut]-NH_2}-NH_2 (SEQ ID NO514 linked to SEQ ID NO:515), exhibits a K_D of 0.79 nM.

Example 14: Enhancing the serum residence of cMet-binding peptides: Conjugation to maleimide

It is known in the art that compounds that contain maleimide and other groups that can react with thiols react with thiols on serum proteins, especially serum albumin, when the compounds are injected. The adducts have serum life times similar to serum albumin, more than 14 days in humans for example.

Methods are available that allow for the direct synthesis of maleimide-labeled linear peptides encompassed by the present invention (Holmes, D. *et al.*, 2000. *Bioconjug. Chem.*, 11:439-444.).

Peptides that include disulfides can be derivatized with maleimide in one of several ways. For example, a third cysteine can be added at the carboxy terminus. The added cysteine is protected with protecting group that is orthogonal to the type of groups used for the cysteines that are to form the disulfide. The disulfide is formed by

selectively deprotecting the intended cysteines and oxidizing the peptide. The final cysteine is then deprotected and the peptide reacted with a large molar excess of a bismaleimide. The resulting compound has one of the maleimides free to react with serum albumin or other thiol-containing serum proteins.

Alternatively, a cyclic peptide of the present invention is synthesized with a lysine-containing C-terminal extention, such as -GGGK (SEQ ID NO:513). Lysines of the cMet-binding motif are protected with ivDde and the C-terminal lysine is deprotected. This lysine is reacted with a maleimide-containing compound, such as N-[e-maleimidocaproyloxy]succinimide ester (Pierce Biotechnology, Rockford, IL) or N-[a-Maleimidoacetoxy]succinimide ester (Pierce Biotechnology).

Example 15: Enhancing the serum residence of cMet-binding peptides: Conjugation to a moiety that binds serum albumin non-covalently

Polypeptides having a molecular weight less than 50-60 kDa are rapidly excreted. Many small molecules, such as fatty acids, bind to serum albumin. Attaching a fatty acid or other serum albumin binding moiety to a peptide causes it to bind non-covalently to serum albumin and can greatly prolong serum residence. Fatty acids attached to peptides of the present invention should contain at least 12 carbons, preferably at least 14 carbons and, more preferably at least 16 carbons. The fatty acid could be straight-chain or branched. The fatty acid could be saturated or unsaturated. Palmate (CH_3-$(CH_2)_{14}$-CO- is a preferred fatty acid. This binding in serum can reduce the rate of excretion (Knudsen, L. *et al.*, 2000. *J. Med. Chem.*, 43:1664-1669). Using methods known in the art, serum-albumin-binding moieties can be conjugated to any one of the peptides or multimeric polypeptide binding constructs herein disclosed. The serum-albumin-binding moiety can be joined to the cMet-binding peptide through a linker. The linker can be peptidic or otherwise, such as PEG. Linkers of zero to about thirty atoms are preferred. It is preferred that the linker be hydrophilic. The serum-albumin-binding moiety can be conjugated to the cMet-binding peptide or construct at either end or though a side group of an appended amino acid. Suitable side groups include lysine and cysteine. Such compounds also can comprise, for example, chelators for radionuclides, or other detectable labels or therapeutic constructs, as

discussed herein. A cMet peptide or construct joined to a serum-albumin-binding moiety will bind cMet.

Example 16: Enhancing the serum residence of cMet-binding peptides: Conjugation to PEG

Attachment of PEG to proteins and peptides enhances the serum residence of these molecules. Attachment of PEG (linear or branched) to a cMet-binding peptide or multimeric polypeptide construct is expected give substantial enhancement of serum residence time. The molecular weight of the PEG be at least 10 kDa, more preferably at least 20 kDa, and most preferably 30 kDa or more. The PEG can be attached at the N- or C-terminus. Methods of attaching PEG to peptides are well known in the art. PEG can be attached to reactive side groups such as lysine or cysteine.

Example 17: Enhancing the serum residence of cMet-binding peptides: fusion to serum protein

Proteins comprising serum albumin (SA) and other proteins have enhanced serum residence times. The amino-acid sequence of human SA (hSA) is shown in Table 10. Table 11 shows a fusion protein comprising of (SEQ ID NO:657), mature hSA, and SEQ ID NO:658. The cMet-binding peptides are separated from mature hSA by linkers that are rich in glycine to allow flexible spacing. One need not use all of hSA to obtain an injectable protein that will have an enhanced serum residence time. Chemical groups, such as maleimide and alpha bromo carboxylates, react with the unpaired cysteine (residue 34) to form stable adducts. Thus, one can attach a single chelator to hSA fusion proteins so that the adduct will bind a radionuclide. One can prepare a chelator with a maleimide group and couple that to hSA or an hSA derivative. Alternatively, hSA or an hSA derivative can be reacted with a bismaleimide and a chelator carrying a reactive thiol could be reacted with the bismaleimide-derivatized hSA.

Construction of genes that encode a given amino-acid sequence are known in the art. Expression of HSA fusions in *Saccharomyces cerevisiae* is known in the art.

Example 18: Pretargeting radioactivity or toxins to cMet expressing tumors

Conventional radioimmune cancer therapy is plagued by two problems. The generally attainable targeting ratio (ratio of administered dose localizing to tumor versus administered dose circulating in blood or ratio of administered dose localizing to tumor versus administered dose migrating to bone marrow) is low. Also, the absolute dose of radiation or therapeutic agent delivered to the tumor is insufficient in many cases to elicit a significant tumor response. Improvement in targeting ratio or absolute dose to tumor would be of great importance for cancer therapy.

The present invention provides methods of increasing active agent localization at a target cell site of a mammalian recipient. The methods include, for example, a) administering to a recipient a fusion protein comprising a targeting moiety and a member of a ligand-anti-ligand binding pair; b) thereafter administering to the recipient a clearing agent capable of directing the clearance of circulating fusion protein via hepatocyte receptors of the recipient, wherein the clearing agent incorporates a member of the ligand-anti-ligand binding pair; and c) subsequently administering to the recipient an active agent comprising a ligand/anti-ligand binding pair member.

It is known in the art that hexoses, particularly the hexoses galactose, glucose, mannose, mannose-6-phosphate, N-acetylglucosamine, pentamannosyl phosphate, N-acetylgalactosamine, thioglycosides of galactose, and mixtures thereof are effective in causing hepatic clearance. Binding of sugars to hepatic receptors is not, however, the only means of directing a molecule to the liver.

> Clearance of carcinoembryonic antigen (CEA) from the circulation is by binding to Kupffer cells in the liver. We have shown that CEA binding to Kupffer cells occurs via a peptide sequence YPELPK representing amino acids 107-112 of the CEA sequence. This peptide sequence is located in the region between the N-terminal and the first immunoglobulin like loop domain. Using native CEA and peptides containing this sequence complexed with a heterobifunctional crosslinking agent and ligand blotting with biotinylated CEA and NCA we have shown binding to an 80kD protein on the Kupffer cell surface. This binding protein may be important in the development of hepatic metastases. (Thomas, P. *et al.*, 1992. *Biochem. Biophys. Res. Commun.*, 188:671-677)

To use YPELPK (SEQ ID NO:655) as a clearance agent, one fuses this sequence via a linker to a moiety that binds the fusion protein (Ab). For example, if the Ab has affinity for DOTA/Re, one would make a derivative having YPELPK attached to DOTA/Re; for example, rvYPELPKpsGGG-DOTA. 'rvYPELPKps' is a fragment of CEA which includes the YPELPK sequence identified by Thomas *et al.* (*supra*). Any convenient point on DOTA can be use for attachment. RVYPELPKPSGGG-DOTA/cold Re (SEQ ID NO:656) would then be used as a clearing agent. The Fab corresponding to the fusion Ab would have affinity for the clearing agent of Kd < 100 nM, preferably Kd < 10 nM, and most preferably Kd < 1 nM.

The therapeutic agent would contain DOTA/^{185}Re. In a preferred embodiment, the therapeutic agent would contain two or more DOTA moieties so that the Ab immobilized on the tumor would bind the bis-DOTA compound with high avidity. The two DOTA moieties would preferably be connected with a hydrophilic linker of ten to thirty units of PEG. PEG is a preferred linker because it is not degraded, promotes solubility. Ten to thirty units of PEG is not sufficient to give the bis DOTA compound a very long serum residence time. A half life of 30 minutes to 10 hours is acceptable. The serum half life should be longer than the radioactive half life of the radionuclide used so that most of the radiation is delivered to the tumor or to the external environment.

In one embodiment, a "fusion protein" of the present invention comprises at least one cMet-binding peptide fused to the amino terminus or the carboxy terminus of either the light chain (LC) or the heavy chain (HC) of a human antibody. Optionally and preferably, two or more cMet-binding peptides are fused to the antibody. The antibody is picked to have high affinity for a small molecule that can be made radioactive or have a toxin attached. Preferably, the affinity of the Fab corresponding to the Ab has affinity for the small molecule with K_d less than 100 nM, more preferably less than 10 nM, and most preferably less than 1 nM. The small molecule could be a chelator capable of binding a useful radioactive atom, many of which are listed herein. The small molecule could be a peptide having one or more tyrosines to which radioactive iodine can be attached without greatly affecting the binding property of the peptide.

Any cMet-binding peptide (CMBP) of the present invention can be fused to either end of either chain of an antibody that is capable of binding a small radioactive compound. Useful embodiments include:

1) CMBP#1::link::LC / HC,
2) LC::link::CMBP#1 / HC,
3) LC / CMBP#1::link::HC,
4) LC / HC::link::CMBP#1,
5) CMBP#1::link1::LC::link2::CMBP#2 / HC,
6) LC / CMBP#1::link1::HC::link2::CMBP#2,
7) CMBP#1::link1::LC / CMBP#2::link2::HC,
8) CMBP#1::link1::LC / HC::link2:: CMBP#2,
9) LC::link1::CMBP#1 / CMBP#2::link2::HC,
10) LC::link1::CMBP#1 / HC::link2:: CMBP#2,
11) CMBP#1::link1::LC::link2::CMBP#2 / CMBP#3::link3::HC,
12) CMBP#1::link1::LC::link2::CMBP#2 / HC::link3::CMBP#3,
13) CMBP#3::link3::LC / CMBP#1::link1::HC::link2::CMBP#2,
14) LC::link3::CMBP#3 / CMBP#1::link1::HC::link2::CMBP#2, and
15) CMBP#1::link1::LC::link2::CMBP#2 / CMBP#3::link3::HC::link4::CMBP#4.

In cases (5)-(15), the linkers (shown as "link1", "link2", "link3", and "link4") can be the same or different or be absent. These linkers, if present, are preferably hydrophilic, protease resistant, non-toxic, non-immunogenic, and flexible. Preferably, the linkers do not contain glycosylation sites or sequences known to cause hepatic clearance. A length of zero to fifteen amino acids is preferred. The cMet-binding peptides (CMBP#1, #2, #3, and #4) could be the same or different. If the encoded amino-acid sequences are the same, it is preferred that the DNA encoding these sequences is different.

Since antibodies are dimeric, each fusion protein will present two copies of each of the fused peptides. In case (15), there will be eight CMBPs present and binding to cMet-displaying cells should be highly avid. It is possible that tumor penetration will be aided by moderate cMet affinity in each of the CMBPs rather than maximal affinity.

The fusion protein is produced in eukaryotic cells so that the constant parts of the HC will be glycosylated. Preferably, the cells are mammalian cells, such as CHO cells.

The fusion proteins are injected into a patient and time is allowed for the fusion protein to accumulate at the tumor. A clearing agent is injected so that fusion protein that has not become immobilized at the tumor will be cleared. In previous pretargeting methods, the antibody combining site has been used to target to the tumor and biotin/avidin or biotin/streptavidin has been used to attach the radioactive or toxic agent to the immobilized antibody. The biotin/avidin or streptavidin binding is essentially irreversible. Here we fuse a target-binding peptide to the antibody which is picked to bind a radioactive or toxic agent. Because the fusion protein contains 2, 4, 6, or 8 CMBPs, binding of the fusion protein to the tumor is very avid. A clearing agent that will cause fusion protein not immobilized at the tumor to clear can be administered between 2 and 48 hours of the injection of the fusion protein. Because the clearance agent is monomeric in the moiety that binds the antibody, complexes of clearance agent and immobilized fusion protein will not have very long life times. Within 4 to 48 hours of injecting clearance agent, the immobilized antibody will have lost any clearance agent that binds there. The active agent is, preferably, dimeric in the moiety that binds the fusion protein. The active agent is injected between 2 and ~ 48 hours of injection of clearance agent.

Example 19: Binding of cMet binding peptides/avidin HRP complex to MDA-MB-231 cells

The spacer length requirements for the binding of a biotinylated derivative of a cMet binding peptide, SEQ ID NO:514, to cMet expressing MDA-MB-231 cells were determined. In order to decide the spacer length to be placed in between peptide and biotin, derivatives were synthesized with no spacer, a single spacer, J, and two spacers, JJ. These three different derivatives of cMet-binding peptide SEQ ID NO:514 and a control peptide that does not bind to cMet, were tested as tetrameric complexes with neutravidin HRP for their ability to bind cMet expressing MB-231 cells. All three tetrameric complexes of cMet-binding peptides bound to the MB231 cells as compared to control peptide; however, the peptide with the JJ spacer exhibited the best K_D (12.62

nM). This suggests that inclusion of two spacers (JJ) between the cMet-binding peptide and the biotin is better than one or no spacer.

Cell Culture: MDA-MB231 cells were obtained from ATCC and grown as monolayer culture in their recommended media plus 1 mL/L pen/strep (InVitrogen, Carlsbad, CA). Cells were split the day before the assay, 35000 cells were added to each well of a 96-well plate.

Binding of peptide/neutravidin HRP to MDA-MB-231 cells

Complexes of control peptide, and the SEQ ID NO:514 derivatives described above, with neutravidin-HRP, were prepared as described above and tested for their ability to bind MDA-MB-231 cells. During the peptide/neutravidin-HRP complex preparation, a 7.5-fold excess of biotinylated peptides over neutravidin-HRP was used to make sure that all four biotin binding sites on neutravidin were occupied. After complex formation, the excess of free biotinylated peptides was removed using soft release avidin-sepharose to avoid any competition between free biotinylated peptides and neutravidin HRP-complexed biotinylated peptides. The experiment was performed at several different concentrations of peptide/neutravidin-HRP, from 0.28 nM to 33.33 nM, to generate saturation binding curves for derivatives without a J spacer and with a single J spacer (FIG. 14), and 0.28 nM to 16.65 nM to generate a saturation binding curve for the derivative with the JJ spacer (FIG. 14). In order to draw the saturation binding curve, the background binding of the control peptide/neutravidin HRP complex was subtracted from the binding of the SEQ ID NO:514 derivatives in complex with neutravidin-HRP for each concentration tested. Therefore, absorbance on the Y-axis of FIG. 14 is differential absorbance (cMet-binding peptide minus control peptide) and not the absolute absorbance. Analysis of the saturation binding data in FIG. using Graph Pad Prism software (version 3.0) yielded a K_D of 12.62 nM (+/-3.16) for the tetrameric derivative with the JJ spacer, 155.4 nM (+/- 86.56) for the tetrameric derivative with the J spacer and 123.8 nM (+/- 37.71) for the tetrameric derivative without a spacer peptide complexes. These binding constants are, as expected, lower than that measured by FP for the related monodentate peptide SEQ ID NO:514 (880 nM).

Results: It is evident from FIG. 14 that the derivative with the JJ spacer showed much better binding to cMet on MDA-MB-231 cells than either of the other two derivatives, with a K_D of 12.62 nM after subtracting binding of control peptide as background binding (n = 1). This suggests that a certain minimum spacer length may be required to be able to reach multiple different binding sites on cells and thus achieve multimeric binding. This minimum spacer length could depend on the spacing between different target molecules on cells. As was the case where the binding target was KDR, the neutravidin-HRP assay with biotinylated peptides identified with phage display was useful for identifying peptides capable of binding to an immobilized target even when the affinity of the monomeric binding sequence is too low for an ELISA-type assay (with washing steps after binding) to work well.

Table 6. cMet-binding peptide sequences

CLASS I

TN6:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:001	571-C05,	GSWIICWWDNCGSSAP
SEQ ID NO:002	465-A06,	GSYYDCREFQCNKPAP
SEQ ID NO:003	465-D09,	GSSHLCNPEFCHFTAP
SEQ ID NO:004	569-H10,	GSMLMCELWWCRFLAP
SEQ ID NO:005	470-E11,	GSLIFCPYGECMMYAP
SEQ ID NO:006	452-F01,	GSEYSCRTSRCIFSAP
SEQ ID NO:007	569-C03,	GSFILCWWTFCDTNAP
SEQ ID NO:008	574-H03,	GSSTICPGTACVDHAP
SEQ ID NO:009	567-C08,	GSLIICWWSWCDKQAP
SEQ ID NO:010	561-C08,	GSFNICPYQWCTLWAP

Consensus Motif: G-S-X1-X2-X3-C-X4-X5-X6-X7-C-X8-X9-X10-A-P-G-G-K; where X1 is F, L, S, W, Y, or M; X2 is I, Y, H, T, or N; X3 is I, L, D, M, F, or S, preferably I; X4 is P,

R, W, N, or E, preferably W or P; X5 is W, Y, E, P, L, T, or G; X6 is S, T, D, F, E, W, G, or Q; X7 is F, W, N, Q, E, R, or A; X8 is G, N, H, R, M, I, D, V, or T; X9 is S, K, F, M, T, D, or L; and X10 is S, P, T, L, Y, N, H, Q, or W.

CLASS II

TN8:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:011	573-F04,	AGGFACGPPWDICWMFGT
SEQ ID NO:012	570-E07,	AGAWNCEYPTFICEWQGA
SEQ ID NO:013	456-E04,	AGNWICNLSEMRCYPKGT
SEQ ID NO:014	434-E12,	AGDGWCMAWPEICEWLGT
SEQ ID NO:015	489-A04,	AGLYLCDLSIMYCFFQGT
SEQ ID NO:016	484-D08,	AGWWSCQWELNVCIWQGT
SEQ ID NO:017	482-D02,	AGYYHCIDDFPQCKWMGT
SEQ ID NO:018	437-A09,	AGWFECEFGFWGCNWLGT
SEQ ID NO:019	352-E04,	AGTVYCSWESSECWWVGT
SEQ ID NO:020	376-E05,	AGVWICRVWDDECFFQGT
SEQ ID NO:021	482-A12,	AGDHYCWEEWWFCWDSGT
SEQ ID NO:022	423-C11,	AGVLQCIGFEWFCDIWGT
SEQ ID NO:023	499-C09,	AGVIVCNLSMMYCLYPGT
SEQ ID NO:024	457-A09,	AGYPECKDNYHWCEWKGT
SEQ ID NO:025	573-E07,	AGWTWCDLSMMSCIFHGT
SEQ ID NO:026	465-F08,	AGVTNCNLSTMFCFLHGT
SEQ ID NO:027	465-E09,	AGTLSCSEEYKSCQLQGT
SEQ ID NO:028	444-B08,	AGTIRCNLAMMVCMFEGT
SEQ ID NO:029	465-E11,	AGQYLCTQAALGCPEWGT
SEQ ID NO:030	465-D12,	AGQMWCAEKNSKCYQWGT
SEQ ID NO:031	470-A02,	AGQAVCEWGPFWCQMQGT
SEQ ID NO:032	465-C01,	AGPYSCHSESHDCKLMGT
SEQ ID NO:033	448-H02,	AGPLFCFEWPSLCHWGGT
SEQ ID NO:034	465-D01,	AGNLPCHWNMSVCDHQGT

SEQ ID NO:035 571-C11, AGMDFCEGFWFLCIGNAT
SEQ ID NO:036 465-B11, AGLLGCWDMPMECTGEGT
SEQ ID NO:037 442-E08, AGKYMCEGFEWFCEMWGT
SEQ ID NO:038 465-C11, AGKTVCQKWESVCSGMGT
SEQ ID NO:039 465-F10, AGKQWCVVWEETCDQLGT
SEQ ID NO:040 471-A11, AGIWFCNNEEKSCWAYGT
SEQ ID NO:041 465-C07, AGHTICQHKALGCPANGT
SEQ ID NO:042 465-D04, AGHFECPKHQYMCDMPGT
SEQ ID NO:043 445-E04, AGGNWCSFYEELCEWLGT
SEQ ID NO:044 465-B06, AGGHWCLELKHLCPPYGT
SEQ ID NO:045 470-C02, AGFWDCGWMMQDCHMHGT
SEQ ID NO:046 458-B05, ADAWMCEYFQWNCGDKGT
SEQ ID NO:047 545-E08, GDGFLCRWENGWCEFWDP

Consensus Motif: A-G-X1-X2-X3-C-X4-X5-X6-X7-X8-X9-C-X10-X11-X12-G-T-G-G-G-K; where

X1 is any amino acid other than C, preferably G, V, W, T, K, Q;

X2 is any amino acid other than C, preferably W, Y, L, F, T;

X3 is any amino acid other than C, preferably W, E, F, I, L, S

X4 is any amino acid other than C, preferably E, N, Q;

X5 is any amino acid other than C, preferably W, L, E;

X6 is any amino acid other than C, preferably E, S, Y;

X7 is any amino acid other than C, preferably E, M, P;

X8 is any amino acid other than C, preferably M, S, W;

X9 is any amino acid other than C, preferably F, L, V;

X10 is any amino acid other than C, preferably E, D, W;

X11 is any amino acid other than C, preferably W, F, M; and

X12 is any amino acid other than C, preferably Q, W, L.

CLASS III

TN9 #1:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:048	325-H05,	AGSIQCKGPPWFSCAMYGT
SEQ ID NO:049	330-F05,	AGYYGCKGPPTFECQWMGT
SEQ ID NO:050	333-F09,	AGQFKCAGPPSFACWMTGT
SEQ ID NO:051	336-G04,	AGWFQCKGPPSFECERHGT
SEQ ID NO:052	334-G06,	AGWTHCIGPPTFECIPMGT
SEQ ID NO:053	330-B07,	AGSFACKGPPTFACVEFGT
SEQ ID NO:054	330-C10,	AGNYFCAGSPSFSCYFMGT
SEQ ID NO:055	331-G04,	AGSWHCAGPPSFECWEFGT
SEQ ID NO:056	548-F06,	AGWISCAGPPTFACWPGGT
SEQ ID NO:057	538-F08,	AGFVNCKGPPTFECILTGT
SEQ ID NO:058	547-H07,	AGDWICHGPPMFECEWVGT
SEQ ID NO:059	323-A11,	AGYTSCVGPPSFECTPYGT
SEQ ID NO:060	333-H03,	AGYFECKGPPTFECWLSGT
SEQ ID NO:061	329-D02,	AGHAWCSGPPRFECWPPGT
SEQ ID NO:062	550-C09,	AGHYWCAGPPTFICMGPGT
SEQ ID NO:063	548-E08,	AGETTCLGWPTFVCVDYGT
SEQ ID NO:064	332-A05,	AGHGTCRGWPTFECIYFGT
SEQ ID NO:065	330-C01,	AGDWHCQGPPAFMCWMIGT
SEQ ID NO:066	545-A09,	AGLPKCSGPPWFSCYYGGT
SEQ ID NO:067	334-C08,	AGGWECTGPPWFQCGYYGT
SEQ ID NO:068	333-C05,	AGDIVCTGHPYFECWSWGT
SEQ ID NO:069	551-B02,	AGTWHCAGPPWFTCYMDGT
SEQ ID NO:070	551-G12,	AGSWECTGPPSFHCQWYGT
SEQ ID NO:071	330-G09,	AGHWICVGPPTFSCQWHGT
SEQ ID NO:072	331-F01,	AGEWWCHGPPEFLCYWTGT
SEQ ID NO:073	274-B07,	AGETVCYWLNGWFCVDDGT
SEQ ID NO:074	335-D11,	AGSIQCVGPPSFECTPYGT
SEQ ID NO:075	336-D07,	AGYSVCKGYPSFECAFFGT
SEQ ID NO:076	332-C03,	AGVNSCLGPPTFECYQMGT

SEQ ID NO:077 331-D03, AGYWHCKGPPHFACEFHGT
SEQ ID NO:078 331-G06, AGNWICTGPPSFGCWYHGT
SEQ ID NO:079 552-G03, AGYWSCAGPPMFMCTWQGT
SEQ ID NO:080 552-G11, AGYWDCKGPPHFFCEWHGT
SEQ ID NO:081 550-G08, AGYFHCSGSPWFQCDYYGT
SEQ ID NO:082 550-G12, AGWYNCSGENFWNCKWIGT
SEQ ID NO:083 552-A01, AGWSDCLGPPQFTCVHWGT
SEQ ID NO:084 548-C06, AGTMYCLGPPTFICQQYGT
SEQ ID NO:085 545-B12, AGSYWCSGPPTFMCRYEGT
SEQ ID NO:086 549-F06, AGSTDCRGHPTFECWGWGT
SEQ ID NO:087 552-F01, AGSSPCKGWPTFECYFYGT
SEQ ID NO:088 547-H12, AGSIACTGWPYFSCIDLGT
SEQ ID NO:089 550-F11, AGQFYCSGPPTFQCIMIGT
SEQ ID NO:090 548-D08, AGPWKCTGPPTFSCIQFGT
SEQ ID NO:091 549-D02, AGNYWCSGPPSFICHAVGT
SEQ ID NO:092 552-F02, AGMTLCAGPPTFECYEVGT
SEQ ID NO:093 545-E04, AGETKCSGPPYFYCWMEGT
SEQ ID NO:094 545-E05, AGETFCVGNPSFECWSWGT
SEQ ID NO:095 547-H03, AGETFCSGWPTFECMQWGT
SEQ ID NO:096 552-G09, AGEIFCVGPPTFTCMWTGT
SEQ ID NO:097 550-A08, AGDFICQGPPSFVCTNIGT
SEQ ID NO:098 550-G07, AGAFFCSGPPTFMCSLYGT
SEQ ID NO:099 551-A05, AGWGWCSGPPMFMCTEYGT
SEQ ID NO:100 548-C10, GSEFECTGWPEFRCYEYAP
SEQ ID NO:101 465-C10, GSILYCINRNDPQCPYTAP

Consensus Motif: G-X1-X2-X3-C-X4-G-X5-P-X6-F-X7-C-X8-X9-X10-G-T; where:

X1 is any amino acid other than C, preferably E, S, Y, or W;

X2 is any amino acid other than C, preferably W, T, or F;

X3 is any amino acid other than C, preferably W, H, or F;

X4 is any amino acid other than C, preferably A,K, S, or T;
X5 is any amino acid other than C, preferably P or W;
X6 is any amino acid other than C, preferably T or S;
X7 is any amino acid other than C, preferably E or S;
X8 is any amino acid other than C, preferably W, Y, or I;
X9 is any amino acid other than C, preferably W, Y, M, or E; and
X10 is any amino acid other than C, preferably Y.

CLASS IV

TN9 #2:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:102	605-G10,	SETRPTEAGDLICSGPPTFICTLYHTEPTE
SEQ ID NO:103	593-C01,	SETRPTQAVRSQCSGPPTFECWYFGTEPTE
SEQ ID NO:104	592-C01,	SETRPTEGGSWYCSGPPAFECWWYGTEPTE
SEQ ID NO:105	591-E01,	SETRPTVASRWHCNGPPTFECWRYGTEPTE
SEQ ID NO:106	590-E01,	SETRPTEAGTFHCSGPPTFECWSYGPKPTE
SEQ ID NO:107	589-B01,	SETRPTEAGSLWCMGPPWFCCVIYGTQPTE
SEQ ID NO:108	607-A02,	SETRPTEAGILHCSGPPTFECWWNYTEPTE
SEQ ID NO:109	590-F01,	SETRPTESGRVHCPGPPWFRCARNGTEPTE
SEQ ID NO:110	589-C01,	SETRPTAAGRILCTGPPWFSCAMYGTEPTE
SEQ ID NO:111	606-B11,	SETRPTEAADWLCSGPPTFECWWFGTEPTE
SEQ ID NO:112	593-E01,	SETRPTQVGRWQCDGPPTFACRSYGTEPTE
SEQ ID NO:113	592-F12,	SETRPTEAGSTKCSGPPTFECWWFDTEPTE
SEQ ID NO:114	590-F07,	SETRPTVAGSWHCSGPPTFECWWYGTEPTE
SEQ ID NO:115	588-D02,	SETRPTEAGRNHCKGPPGFRCAMTDTEPTE
SEQ ID NO:116	607-H09,	SETRPTETDFVYCRGPPTFECWWYGTEPTE
SEQ ID NO:117	590-H01,	SETRPTSSGSRHCKGPPTFECWGYGTEPTE
SEQ ID NO:118	589-F01,	SETRPTEAGSWRCSGPPTFECWWYETSPTE
SEQ ID NO:119	608-F11,	SETRPTDAIRSYCSGPPTFECWWFGTEPTE
SEQ ID NO:120	606-D11,	SETRPTEAGSWNCSGPPAFECWWYGSEPTE

SEQ ID NO:121	604-D04,	SETRPTEAGSWQCSGPPTFECWSFGTEPTE
SEQ ID NO:122	602-A11,	SETRPTEAGSWHCNGPPTFECWWYDMEPTE
SEQ ID NO:123	593-F02,	SETRPTEAGRVSCLGPPTFECWWFVPEPTE
SEQ ID NO:124	591-H05,	SETRPTDAGSWRCAGPPTFECWWFGTEPTE
SEQ ID NO:125	590-H06,	SETRPTEPVTWQCTGPPTFECWWLGTEPTE
SEQ ID NO:126	588-F10,	SETRPTDAVSTHCNGPPTFECYIYGTEPTE
SEQ ID NO:127	608-G03,	SETRPTVAESWYCVGPPSFECWWYGTEPTE
SEQ ID NO:128	604-D09,	SETRPTEAGSWNCSGPPTFECWSYQTEPTE
SEQ ID NO:129	602-A12,	SETRPTEAGSGHCNGPPTFKCWWYDMEPTE
SEQ ID NO:130	592-G11,	SETRPTDQDSWQCSGPPTFECWWYGTEPTE
SEQ ID NO:131	588-G01,	SETRPTESTQVQCAGPPSFACWMTGTEPTE
SEQ ID NO:132	606-E05,	SETRPTEVESWHCSGPPTFECWWYGTEPTE
SEQ ID NO:133	594-C07,	SETRPTEAGSFHCSGPPTFECWLYWTDPTE
SEQ ID NO:134	592-H01,	SETRPTEAGQFGCKGPPPFECKLMGRVPTE
SEQ ID NO:135	605-C05,	SETRPTDTVTWHCNGPPTFECWWYGTEPTE
SEQ ID NO:136	594-E08,	SETRPTEADRWHCDGPPTFECWWYGTEPTE
SEQ ID NO:137	593-B11,	SETRPTEAGSIQCVGPPWFSCRMYVTEPTE
SEQ ID NO:138	590-C01,	SETRPTVSGSWQCVGPPTFECWSYGTEPTE
SEQ ID NO:139	612-G11,	SETRPTENGSWHCNGPPTFECWWYGTEPTE
SEQ ID NO:140	612-E08,	SETRPTEAGSWHCSGPPIFECWWYDMEPTE
SEQ ID NO:141	612-A02,	SETRPTVDGGWHCNGPPTFECWMYGTEPTE
SEQ ID NO:142	611-G01,	SETRPTDAGTWNCTGPPSFECWWFGTEPTE
SEQ ID NO:143	610-G04,	SETRPTWDGKWHCSGPPTFECWWYGTEPTE
SEQ ID NO:144	610-E06,	SETRPTEAGSWRCSGPPTFECWWYYTEPTE
SEQ ID NO:145	610-C06,	SETRPTEAGNWLCSGPPTFECWWYVTGPTE
SEQ ID NO:146	610-A04,	SETRPTEGGNWHCSGPPTFECWLYGTEPTE
SEQ ID NO:147	612-D02,	SETRPTEAGGWHCSGPPTFECWWFNMEPTE
SEQ ID NO:148	612-A12,	SETRPTEVISWHCSGPPTFECYRYGTEPTE
SEQ ID NO:149	611-D03,	SETRPTEVGSWHCNGPPTFECWWYGTEPTE
SEQ ID NO:150	610-G10,	SETRPTLASTWYCSGPPTFECWWYGTEPTE
SEQ ID NO:151	610-A11,	SETRPTEAGGWYCKGPPTFECWWDGTEPTE
SEQ ID NO:152	612-H02,	SETRPTEAGGWFCSGPPTFECWWYDTVPTE

SEQ ID NO:153 612-B01, SETRPTEAATWQCSGPPTFECWGYGTEPTE
SEQ ID NO:154 610-C12, SETRPTEAGDYVCVGPPTFECYLMDAEPTE
SEQ ID NO:155 610-B01, SETRPTEAGGWYCSGPPSFECWSYGTEPTE
SEQ ID NO:156 612-H04, SETRPTESSSWHCSGPPTFECWRFGTEPTE
SEQ ID NO:157 612-B09, SETRPTEAGSWYCSGPPTFECWWYYAEPTE
SEQ ID NO:158 611-G07, SETRPTLAGNWQCSGPPTFECWWYGTEPTE
SEQ ID NO:159 611-E10, SETRPTEAGSWHCNGPPTFECWQYGTEPTE
SEQ ID NO:160 610-H02, SETRPTEAGSWECHGPPSFECWWYGTEPTE
SEQ ID NO:161 610-D03, SETRPTEAGSWRCSGPPTFECWWYDAEPTE
SEQ ID NO:162 610-B03, SETRPTEAGSWNCAGPPTFECWWYGTEPTE
SEQ ID NO:163 612-H05, SETRPTEAGSFYCSGPPTFECWQYVPEPTE
SEQ ID NO:164 612-F05, SETRPTEAGSWMCSGPPTFECWQYFTEPTE
SEQ ID NO:165 612-B10, SETRPTEAGSLHCSGPPTFECWWWETEPTE
SEQ ID NO:166 611-E11, SETRPTEEGVWHCNGPPTFECWWYGTEPTE
SEQ ID NO:167 610-F08, SETRPTEAGRWNCSGPPTFECWWYSTEPTE
SEQ ID NO:168 610-D05, SETRPTEAGSWRCSGPPTFECWWFGTEPTE
SEQ ID NO:169 610-B04, SETRPTQAVSSYCSGPPTFECWSFGTEPTE
SEQ ID NO:170 612-B12, SETRPTEAGRSYCSGPPTFECWWYATEPTE
SEQ ID NO:171 611-H01, SETRPTVVAKVHCAGPPTFECWTYGTEPTE
SEQ ID NO:172 610-H05, SETRPTEPGSWHCSGPPTFVCWWWGTEPTE
SEQ ID NO:173 610-F10, SETRPTEAGRWHCSGPPTFECWWHDTEPTE
SEQ ID NO:174 612-H07, SETRPTEAGSWQCTGPPTFECWGYVEEPTE
SEQ ID NO:175 612-G09, SETRPTEAGSWQCGGPPTFECWWYYTGPTE
SEQ ID NO:176 612-F08, SETRPTEAGSWYCTGPPTFECWLYETYPTE
SEQ ID NO:177 611-H08, SETRPTAAWSGSCSGPPSFECWNYGTEPTE
SEQ ID NO:178 610-E01, SETRPTEAGSWQCSGPPTFACWWYGTEPTE
SEQ ID NO:179 610-B09, SETRPTEAGILHCSGPPTFECWWEVMEPTE
SEQ ID NO:180 612-E07, SETRPTEAGRVACSGPPTFECWSYDEEPTE
SEQ ID NO:181 612-C11, SETRPTEAGNWECQGPPTFECWWFGTEPTE
SEQ ID NO:182 610-E04, SETRPTLASNGYCNGPPTFECWHYGTEPTE
SEQ ID NO:183 610-B12, SETRPTEAGSFHCSGPPTFECIWYGSEPTE
SEQ ID NO:184 616-B11, SETRPTEAGSWYCSGPPTFACWWDGTEPTE

SEQ ID NO:185	615-H08,	SETRPTQGDNWNCSGPPTFECWWYGTEPTE
SEQ ID NO:186	615-B11,	SETRPTEAGRWHCNGPPTFECWRYDYDPTE
SEQ ID NO:187	614-C07,	SETRPTEAYSWECTGPPMFECWWYGTEPTE
SEQ ID NO:188	613-H12,	SETRPTEVVDWHCSGPPQFECWWYGTEPTE
SEQ ID NO:189	613-F02,	SETRPTEAGSWNCSGPPTFECWWYGSEPTE
SEQ ID NO:190	613-D05,	SETRPTASGSWHCSGPPTFECWIFGTEPTE
SEQ ID NO:191	612-H12,	SETRPTEAGAWYCMGPPTFECWWYDRGPTE
SEQ ID NO:192	616-D05,	SETRPTEAGGLHCSGPPTFECWWYDTEPTE
SEQ ID NO:193	615-C01,	SETRPTVGGSWDCKGPPTFECWSYGTEPTE
SEQ ID NO:194	614-E09,	SETRPTEAGAWSCLGPPTFECWWYGTEPTE
SEQ ID NO:195	614-A03,	SETRPTEAGSLHCSGPPTFECWWFDTEPTE
SEQ ID NO:196	616-C02,	SETRPTAGRSWECSGPPTFECWVFGTEPTE
SEQ ID NO:197	615-C04,	SETRPTDNGSWHCNGPPTFECWWYGTEPTE
SEQ ID NO:198	614-C12,	SETRPTEAGSWQCKGPPTFECWWYGTEPTE
SEQ ID NO:199	615-C11,	SETRPTEVGNYKCSGPPTFECWWYGTEPTE
SEQ ID NO:200	614-H08,	SETRPTEAGSWHCVGPPTFECWGYVTEPTE
SEQ ID NO:201	614-E11,	SETRPTEAGSFVCKGPPTFECYWFGQDPTE
SEQ ID NO:202	616-E10,	SETRPTEAGSWHCSGPPTFECWWYGPDPTE
SEQ ID NO:203	615-D02,	SETRPTEAERWHCSGPPTFECWWYGTEPTE
SEQ ID NO:204	614-F04,	SETRPTEAGSWHCSGPPTFECWFYVKEPTE
SEQ ID NO:205	614-D06,	SETRPTEAGSWDCSGPPTFECWWFGTEPTE
SEQ ID NO:206	614-B08,	SETRPTEPAGWECRGPPSFECLWYGTEPTE
SEQ ID NO:207	613-H01,	SETRPTDAGPWNCTGPPSFECWWYGTEPTE
SEQ ID NO:208	613-E04,	SETRPTEARGWHCSGPPTFECWLWGTEPTE
SEQ ID NO:209	613-B08,	SETRPTEAGRWNCSGPPTFECWQYEMDPTE
SEQ ID NO:210	615-D04,	SETRPTEAGSWYCSGPPTFECFWYDTEPTE
SEQ ID NO:211	615-A05,	SETRPTESGSWHCSGPPTFECWWFGTEPTE
SEQ ID NO:212	614-E04,	SETRPTEAGSWLCTGPPTFECWWFDTDPTE
SEQ ID NO:213	613-E06,	SETRPTEPSHWHCVGPPTFACWWYVTDPTE
SEQ ID NO:214	613-C05,	SETRPTEAGSWYCSGPPMFECYLFVTEPTE
SEQ ID NO:215	616-C07,	SETRPTEAVNWHCLGPPSFECWQFGTEPTE
SEQ ID NO:216	615-G02,	SETRPTEAGSWHCSGPPTFECWWYGTDPTE

SEQ ID NO:217	615-E06,	SETRPTEAGSWHCSGPPTFECWSFVSLPTE
SEQ ID NO:218	615-A08,	SETRPTEGSEWSCIGPPSFECWWYGTEPTE
SEQ ID NO:219	614-G01,	SETRPTEDGYWNCSGPPTFECWWHGTEPTE
SEQ ID NO:220	613-D01,	SETRPTEAGSWSCSGPPTFECWPYYTEPTE
SEQ ID NO:221	614-G02,	SETRPTEAGSWYCSGPPTFECWWYWPEPTE
SEQ ID NO:222	614-E06,	SETRPTDDGRWSCAGPPTFECWRYGTEPTE
SEQ ID NO:223	620-E11,	SETRPTEGGSWSCGGPPTFECWWFGTEPTE
SEQ ID NO:224	620-A11,	SETRPTVTGSWYCSGPPTFECWWYGTEPTE
SEQ ID NO:225	618-F04,	SETRPTEASSWYCTGPPAFECWWYGTEPTE
SEQ ID NO:226	617-G06,	SETRPTEAGSWLCSGPPTFECWWYGTEPTE
SEQ ID NO:227	616-G06,	SETRPTESVRWYCSGPPTFECWWYGTEPTE
SEQ ID NO:228	620-F10,	SETRPTEAGRLVCSGPPTFMCRTYATDPTE
SEQ ID NO:229	619-G04,	SETRPTEAGSWECTGPPWFVCRQYAIEPTE
SEQ ID NO:230	618-F12,	SETRPTEAGYLYCSGPPTFECWWYDTMPTE
SEQ ID NO:231	618-B06,	SETRPTEAGSWHCSGPPTFECWWFGTEPTE
SEQ ID NO:232	617-E09,	SETRPTEAGNWHCLGPPTFECWWYGTEPTE
SEQ ID NO:233	616-F10,	SETRPTEAGSWHCSGPPTFECWWYDTEPTE
SEQ ID NO:234	620-B11,	SETRPTESGGWYCSGPPAFECWWYGTEPTE
SEQ ID NO:235	619-G07,	SETRPTVAGAVSCSGPPTFECWWYGTEPTE
SEQ ID NO:236	619-E11,	SETRPTEAGRWYCSGPPTFECWWFLPDPTE
SEQ ID NO:237	619-B12,	SETRPTEAGGWHCSGPPSFECWWFDTVPTE
SEQ ID NO:238	618-G11,	SETRPTGVGGWYCSGPPSFECWLYGTEPTE
SEQ ID NO:239	618-B11,	SETRPTQADYLHCSGPPTFECFWYGTEPTE
SEQ ID NO:240	617-F01,	SETRPTGDGNWHCNGPPTFECWRFGTEPTE
SEQ ID NO:241	617-B01,	SETRPTEASNYHCIGPPTFECFWYGTEPTE
SEQ ID NO:242	616-G12,	SETRPTEAGDWLCKGPPTFECWWQVTDPTE
SEQ ID NO:243	620-G01,	SETRPTEAGSWHCNGPPTFECWWYSSDPTE
SEQ ID NO:244	620-C10,	SETRPTEDGGWRCSGPPTFECWWYGTEPTE
SEQ ID NO:245	619-G09,	SETRPTEAGRIECKGPPWFSCVIYGTEPTE
SEQ ID NO:246	619-F06,	SETRPTGGGSWNCSGPPTFECWWYGTEPTE
SEQ ID NO:247	618-C03,	SETRPTEAGSLYCSGPPTFECWWYITHPTE
SEQ ID NO:248	617-F02,	SETRPTEAGRWHCSGPPRFECWWYDTEPTE

SEQ ID NO:249 616-H01, SETRPTEYGSWHCSGPPTFECWYHGTEPTE
SEQ ID NO:250 618-D01, SETRPTEAGNWHCSGPPSFECWWYATEPTE
SEQ ID NO:251 617-F03, SETRPTEQGSWHCKGPPTFECWSYGTEPTE
SEQ ID NO:252 616-H03, SETRPTDAANYHCSGPPTFECWWYGTEPTE
SEQ ID NO:253 616-G02, SETRPTEAGSWYCSGPPMFECWWLAEEPTE
SEQ ID NO:254 620-G09, SETRPTEAGGWYCSGPPAFECWWYATEPTE
SEQ ID NO:255 620-D12, SETRPTEAGIWSCSGPPTFECWWYESSPTE
SEQ ID NO:256 619-A09, SETRPTEEGLRVCSGPPTFECWWYGTEPTE
SEQ ID NO:257 618-D06, SETRPTEAGSWLCFGPPTFECWSFGTEPTE
SEQ ID NO:258 617-H12, SETRPTVAGSWDCSGPPTFECWWYGTEPTE
SEQ ID NO:259 616-H05, SETRPTKADNWHCSGPPTFECWWYGTEPTE
SEQ ID NO:260 619-H10, SETRPTEAGIVYCSGPPTFECWWFGTEPTE
SEQ ID NO:261 619-D03, SETRPTEAGYWHCLGPPTFECWWYVKEPTE
SEQ ID NO:262 618-D12, SETRPTEPGLLHCSGPPTFECWWYGTEPTE
SEQ ID NO:263 620-E04, SETRPTEASSWYCSGPPSFECWWYGTEPTE
SEQ ID NO:264 620-A05, SETRPTEAGSWHCLGPPTFECWWYVKEPTE
SEQ ID NO:265 619-D04, SETRPTEAGIILCKGPPWFSCDIYDTGPTE
SEQ ID NO:266 618-A11, SETRPTAAGNWHCSGPPTFECWAYGTEPTE
SEQ ID NO:267 617-D07, SETRPTVGGSWYCSGPPTFECWSYGTEPTE
SEQ ID NO:268 627-A10, SETRPTEDGWLDCKGPPTFECWWYGTEPTE
SEQ ID NO:269 626-H02, SETRPTEDGNWHCSGPPTFECWSYGTEPTE
SEQ ID NO:270 626-F06, SETRPTEAGSWHCSGPPTFECWYYWPEPTE
SEQ ID NO:271 624-D02, SETRPTEAGSLYCSGPPMFECWWYDWYPTE
SEQ ID NO:272 622-D09, SETRPTEAGGWYCMGPPAFECWWYASEPTE
SEQ ID NO:273 621-F11, SETRPTNAGSWYCSGPPTFECWWYGTEPTE
SEQ ID NO:274 621-B11, SETRPTEASRWHCNGPPTFECWWYGTEPTE
SEQ ID NO:275 627-B03, SETRPTEAGSFVCSGPPTFECWWYNTGPTE
SEQ ID NO:276 626-H03, SETRPTEAGSWHCSGPPTFECWSYGTEPTE
SEQ ID NO:277 626-F07, SETRPTESDIWLCSGPPTFECWWYGTEPTE
SEQ ID NO:278 626-D02, SETRPTDADPWHCSGPPTFECWWFGTEPTE
SEQ ID NO:279 625-B03, SETRPTEAGVVLCSGPPTFECWWYDTEPTE
SEQ ID NO:280 622-D10, SETRPTEVGSVHCSGPPTFECWWFGTEPTE

SEQ ID NO:281 621-G02, SETRPTEAGRWLCSGPPTFECWEYDTEPTE
SEQ ID NO:282 621-E04, SETRPTDAGWLQCSGPPTFECWWYGTEPTE
SEQ ID NO:283 621-B12, SETRPTEASRRHCNGPPTFECWRYGTEPTE
SEQ ID NO:284 626-H04, SETRPTEAGRWYCSGPPTFECWLFVEEPTE
SEQ ID NO:285 626-F11, SETRPTAADSWQCSGPPTFECWSFGTEPTE
SEQ ID NO:286 626-D03, SETRPTEAGSWHCGGPPTFECWMYVTEPTE
SEQ ID NO:287 626-A02, SETRPTDDGSWYCSGPPTFECWWYGTEPTE
SEQ ID NO:288 623-E07, SETRPTEAGYWHCLGPPTFECWWYDMEPTE
SEQ ID NO:289 622-G09, SETRPTEAGILRCSGPPTFECWYYETEPTE
SEQ ID NO:290 622-E05, SETRPTEDVSVHCAGPPTFECWLYGTEPTE
SEQ ID NO:291 622-B12, SETRPTEEGVFQCVGPPTFECWWYGTEPTE
SEQ ID NO:292 621-G07, SETRPTEDGGFFCSGPPTFECWWYGTEPTE
SEQ ID NO:293 621-E07, SETRPTEPGSWHCSGPPTFECWWYGTEPTE
SEQ ID NO:294 621-C01, SETRPTEAGSWHCSGPPTFECWWYDRAPTE
SEQ ID NO:295 626-A05, SETRPTEAGTWYCSGPPTFECWYYATEPTE
SEQ ID NO:296 623-G02, SETRPTEAGSLYCSGPPAFECYWYGTVPTE
SEQ ID NO:297 622-H11, SETRPTDPGVLHCSGPPTFECWWFGTEPTE
SEQ ID NO:298 622-C04, SETRPTEAGTWYCLGPPTFECWSFWQDPTE
SEQ ID NO:299 621-G11, SETRPTEAGRWGCSGPPTFECWWYVAEPTE
SEQ ID NO:300 621-C07, SETRPTEAGIWHCAGPPTFICWLYETEPTE
SEQ ID NO:301 627-C03, SETRPTEAGSWHCSGPPSFECWQYSTEPTE
SEQ ID NO:302 626-D12, SETRPTEAGSWQCSGPPTFECWVYETEPTE
SEQ ID NO:303 626-A06, SETRPTEAGSWYCSGPPTFECWWYDVGPTE
SEQ ID NO:304 623-H02, SETRPTDEVSWECRGPPTFECWWYGTEPTE
SEQ ID NO:305 623-B05, SETRPTEGGSWVCSGPPTFECWWYGTEPTE
SEQ ID NO:306 622-E10, SETRPTEYGSWYCSGPPTFECWWLGTEPTE
SEQ ID NO:307 622-C06, SETRPTEAGVWLCSGPPTFECWWYDTDPTE
SEQ ID NO:308 621-H03, SETRPTMAGSYYCSGPPTFECWVYGTEPTE
SEQ ID NO:309 621-E11, SETRPTEAGYVQCYGPPSFVCHPMVPDPTE
SEQ ID NO:310 621-C08, SETRPTEDGFVLCKGPPWFSCEMYGTEPTE
SEQ ID NO:311 627-C04, SETRPTEAGGWNCSGPPTFECWWYVTEPTE
SEQ ID NO:312 626-A07, SETRPTEDGSWECFGPPTFECWSYGTEPTE

SEQ ID NO:313 623-H08, SETRPTDAVSYVCKGPPTFECWWYGTEPTE
SEQ ID NO:314 622-F05, SETRPTEARSWHCSGPPTFECWWYGTEPTE
SEQ ID NO:315 627-A04, SETRPTASVSWHCSGPPTFECWSYGTEPTE
SEQ ID NO:316 626-G05, SETRPTEAGSWYCSGPPTFECWYYDMDPTE
SEQ ID NO:317 623-H11, SETRPTEAGSWLCSGPPTFECWWFGTEPTE
SEQ ID NO:318 622-F11, SETRPTGDGSWYCSGPPTFECWWLGTEPTE
SEQ ID NO:319 621-F03, SETRPTEAGSWYCSGPPTFECWWYFLDPTE
SEQ ID NO:320 626-F01, SETRPTEAGGWYCSGPPTFECWWFATEPTE
SEQ ID NO:321 621-F04, SETRPTEAGDLDCLGPPTFICRIYGTEPTE
SEQ ID NO:322 630-F06, SETRPTEAGSWQCVGPPTFECWSFGTEPTE
SEQ ID NO:323 630-A03, SETRPTEADSWYCSGPPTFECWLFGTEPTE
SEQ ID NO:324 629-F10, SETRPTQADSWYCSGPPTFECWWWGTEPTE
SEQ ID NO:325 629-D11, SETRPTEAFSWDCSGPPTFECWWFGTEPTE
SEQ ID NO:326 629-B06, SETRPTEAGSWQCSGPPVFECWWYDTEPTE
SEQ ID NO:327 628-H01, SETRPTEAGNVQCSGPPTFECWWFDTEPTE
SEQ ID NO:328 628-F03, SETRPTEAGSVVCSGPPRFECWAFVTEPTE
SEQ ID NO:329 627-G02, SETRPTEDGTLHCSGPPTFACWWYGTEPTE
SEQ ID NO:330 629-E01, SETRPTDAEVWVCNGPPTFECWWYGTEPTE
SEQ ID NO:331 628-H09, SETRPTEDVTFHCSGPPTFECWLYGTEPTE
SEQ ID NO:332 628-A05, SETRPTSDFDWHCKGPPTFECWSYGTEPTE
SEQ ID NO:333 627-G04, SETRPTEADSWYCSGPPTFECWWYVPEPTE
SEQ ID NO:334 630-A05, SETRPTDDGNWYCSGPPTFECWWYGTEPTE
SEQ ID NO:335 629-E03, SETRPTEAGSWYCSGPPTFECWRYDTDPTE
SEQ ID NO:336 629-C02, SETRPTEAGPWSCSGPPTFECWWFDTEPTE
SEQ ID NO:337 628-H10, SETRPTEAGMFLCSGPPAFECWWYDTEPTE
SEQ ID NO:338 628-F12, SETRPTEAGSLYCSGPPTFECWLYDVEPTE
SEQ ID NO:339 627-D12, SETRPTEAGQWNCSGPPTFECWWYDIEPTE
SEQ ID NO:340 630-G02, SETRPTEAGSWYCSGPPTFECWWFETEPTE
SEQ ID NO:341 629-E06, SETRPTEAGSFVCSGPPTFECWGYVTEPTE
SEQ ID NO:342 628-D07, SETRPTQDGTWFCSGPPTFECWWYGTEPTE
SEQ ID NO:343 627-E06, SETRPTEGDSWHCAGPPTFECWWYGTEPTE
SEQ ID NO:344 629-E07, SETRPTEAGSWSCSGPPTFECWSYGTEPTE

SEQ ID NO:345 629-C11, SETRPTEAGRIQCSGPPTFECWWYDEEPTE
SEQ ID NO:346 629-A03, SETRPTEAGTIVCKGPPWFSCEIYETEPTE
SEQ ID NO:347 628-A12, SETRPTEAGDWYCSGPPAFECWEYLGEPTE
SEQ ID NO:348 627-E08, SETRPTEAGSWFCSGPPSFECWSYVTEPTE
SEQ ID NO:349 629-E08, SETRPTEAGSWHCSGPPAFECWWYDNEPTE
SEQ ID NO:350 629-B02, SETRPTEAGRWTCSGPPTFECWWYVSDPTE
SEQ ID NO:351 628-E06, SETRPTEAGEWYCGGPPTFECWWFDTAPTE
SEQ ID NO:352 627-G09, SETRPTEAGSWHCSGPPSFECWWFDTGPTE
SEQ ID NO:353 631-A11, SETRPTEAGSFICSGPPTFECWWYGTEPTE
SEQ ID NO:354 630-C10, SETRPTEDVRWYCSGPPTFECWWFGTEPTE
SEQ ID NO:355 628-B08, SETRPTEAGSWYCSGPPTFECWWYVPEPTE
SEQ ID NO:356 629-F03, SETRPTEAGNWLCSGPPAFECWWFVAEPTE
SEQ ID NO:357 632-A09, SETRPTEAGSWYCSGPPTFECWWYGTEPTE
SEQ ID NO:358 632-G07, SETRPTEAGDWLCAGPPTFECWWWGTDPTE
SEQ ID NO:359 631-F12, SETRPTEAGSWHCVGPPTFECWWFDTEPTE
SEQ ID NO:360 633-A02, SETRPTEAGEWSCSGPPTFECWWWDMEPTE
SEQ ID NO:361 633-B06, SETRPTYYVSWYCSGPPTFECWSYGTEPTE
SEQ ID NO:362 632-D11, SETRPTEDGSWYCSGPPTFECWWYGTEPTE
SEQ ID NO:363 631-D10, SETRPTEDGTWYCSGPPTFECWWYGTEPTE
SEQ ID NO:364 633-F09, SETRPTETDSWVCSGPPTFECWWYGTEPTE

Consensus Motif#1: G-X1-X2-X3-C-X4-G-P-P-X5-F-X6-C-X7-X8-X9-X10-X11-X12-P-T-E, where:

X1 is any amino acid other than C, preferably S, R, I, D, or N;

X2 is any amino acid other than C, preferably W, L, F, V, or I;

X3 is any amino acid other than C, preferably H, Y, L, Q, N, or V;

X4 is any amino acid other than C, preferably S, K, or L;

X5 is any amino acid other than C, preferably T, S, A, or W;

X6 is any amino acid other than C, preferably E or S;
X7 is any amino acid other than C, preferably W;
X8 is any amino acid other than C, preferably W, S, or L;
X9 is any amino acid other than C, preferably Y or F;
X10 is any amino acid other than C, preferably D, G, V, or E;
X11 is any amino acid other than C, preferably T, P, M, or S; and
X12 is any amino acid other than C, preferably E or G.

Motif#2: T-X1-X2-X3-X4-X5-X6-C-X7-G-P-P-X8-F-X9-C-X10-X11-X12-G, where:
X1 is any amino acid other than C, preferably E, D, or V;
X2 is any amino acid other than C, preferably A, D, G, S, or V;
X3 is any amino acid other than C, preferably G, V, D, or S;
X4 is any amino acid other than C, preferably S, N, R, T, or G;
X5 is any amino acid other than C, preferably W;
X6 is any amino acid other than C, preferably H or Q;
X7 is any amino acid other than C, preferably S, N, or K;
X8 is any amino acid other than C, preferably T;
X9 is any amino acid other than C, preferably E;
X10 is any amino acid other than C, preferably W;
X11 is any amino acid other than C, preferably W or S; and
X12 is any amino acid other than C, preferably Y or F.

CLASS V

TN10:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:365	545-C02,	GSWRFCGGEYSFQVCQDVAP

SEQ ID NO:366 546-E02, GSHHTCLDGFAGWRCTEVAP
SEQ ID NO:367 545-C11, GSFAPCGWPSFAIDCIAEAP
SEQ ID NO:368 549-G01, GSTKVCHEKWNQLFCHNQAP
SEQ ID NO:369 548-F07, GSPEMCMMFPFLYPCNHHAP
SEQ ID NO:370 551-H10, GSFFPCWRIDRFGYCHANAP

Consensus Motif: S-X1-X2-X3-C-X4-X5-X6-X7-X8-X9-X10-X11-C-X12-X13-X14-A-P, where
X1 is one of W, H, F, T, or P;
X2 is one of R, H, A, K, E, or F;
X3 is one of F, T, P, V, or M;
X4 is one of F, L, H, M, or W;
X5 is one of G, D, W, E, M, or R;
X6 is one of E, G, P, K, F, or I;
X7 is one of Y, F, S, W, P, or D;
X8 is one of S, A, F, N, or R;
X9 is one of F, G, A, Q, or L;
X10 is one of Q, W, I, L, Y, or G;
X11 is one of V, R, D, F, P, or Y;
X12 is one of Q, T, I, H, or N;
X13 is one of D, E, A, N, or H; and
X14 is one of V, E, Q, H, or N

CLASS VI
TN11 #1:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:371	443-H10,	GSQQICDRKEYRFQACLSDAP
SEQ ID NO:372	557-A12,	GSTMSCWRWGRDAYSCNQMAP
SEQ ID NO:373	465-A03,	GSSQICAVYLDDTHNCERHAP
SEQ ID NO:374	446-E12,	GSSHCNQMITPWQNCGMRAP
SEQ ID NO:375	445-E06,	GSSARCDELINDFHSCLVMAP
SEQ ID NO:376	452-A03,	GSRFHCWQGDLMQTYCMPMAP

SEQ ID NO:377 465-C06, GSQNNCEYGSRGSSFCLAMAP
SEQ ID NO:378 441-H01, GSMNMCDTTDEISPTCHPSAP
SEQ ID NO:379 443-D04, GSMLGCLFEHQNKYDCYVLAP
SEQ ID NO:380 445-G12, GSLYRCLGEASPTPPCAYEAP
SEQ ID NO:381 442-E03, GSGMGCHQVNISTGDCAEDAP
SEQ ID NO:382 453-A05, GSGDPCSPGPSINGHCSVMAP
SEQ ID NO:383 445-E07, GSFWNCTTDLGAMSDCGFFAP
SEQ ID NO:384 451-B12, GSFTACNKTSTTRQPCNPYAP
SEQ ID NO:385 465-B07, GSELFCFYHHQGYEGCDVLAP
SEQ ID NO:386 451-C06, GSDMNCTVLAQDQIFCFREAP
SEQ ID NO:387 445-E11, GSAGWCYTMNYVDQLCTYMAP

Consensus Motif: S-X1-X2-X3-C-X4-X5-X6-X7-X8-X9-X10-X11-X12-C-X13-X14-X15-A-P, where

X1 is any amino acid other than C, preferably S, F, G, M, or Q;

X2 is any amino acid other than C, preferably M, L, N, or Q;

X3 is any amino acid other than C, preferably N, G, H, I, or R;

X4 is any amino acid other than C, preferably D, L, N, T, or W;

X5 is any amino acid other than C, preferably Q, T, R, V, or Y;

X6 is any amino acid other than C, preferably G, E, L, M, or T;

X7 is any amino acid other than C, preferably A, D, H, I, L, N, or S;

X8 is any amino acid other than C, preferably Q, R, S, T, or Y;

X9 is any amino acid other than C, preferably D, G, I, or P;

X10 is any amino acid other than C, preferably T, F, or Q;
X11 is any amino acid other than C, preferably Q, F, H, P, S, or Y;
X12 is any amino acid other than C, preferably D, F, N, P, or S;
X13 is any amino acid other than C, preferably L, A, G, N, or S;
X14 is any amino acid other than C, preferably V, P, R, or Y; and
X15 is any amino acid other than C, preferably M, D, E, or L.

CLASS VII

TN11 #2

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:388	593-G11,	SETRPTEAGMCACRGPPAFVCQWYGSEPTE
SEQ ID NO:389	631-E12,	SETRPTEAGSCHCSGPPTFECWSYVTEPTE

CLASS VIII

TN12:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:390	546-G02,	GDYDYCDFDLETYIPECHSYDP
SEQ ID NO:391	333-C03,	GDDFHCEFIDDYQSEICYFNDP
SEQ ID NO:392	549-G05,	GDLLVCKFDDKFWTETCEWADP
SEQ ID NO:393	546-B01,	GDSYNCSWDSKTFEVTCLYADP
SEQ ID NO:394	551-D02,	GDASWCDENSPAAWFYCELWDP
SEQ ID NO:395	334-F05,	GDLLGCGYQEKGGEYKCRFNDP
SEQ ID NO:396	330-G02,	GDPWWCFEKDSFIPFACWHHDP
SEQ ID NO:397	316-F08,	GDYYQCQFSKDMYSERCWPYDP
SEQ ID NO:398	332-H09,	GDNRFCSWVYNVDDWWCVDNDP
SEQ ID NO:399	545-H12,	GDYSECFFEPDSFEVKCYDRDP
SEQ ID NO:400	548-G05,	GDYRMCQISDMWGNYECSSDDP

SEQ ID NO:401 547-C09, GDPDECQLNRETFEVWCPWHDP

SEQ ID NO:402 545-F04, GDHRKCEISAKTHEVTCYDNDP

SEQ ID NO:403 552-F06, GDHLTCEFRDDGWKEHCWWSDP

SEQ ID NO:404 531-E11, GDASMCYDGLALRWDQCWPHDP

Consensus Motif: D-X1-X2-X3-C-X4-X5-X-6-X7-X8-X9-X10-X11-X12-X13-C-X14-X15-X16-D-P, where

X1 is any amino acid other than C, preferably Y, A, H, L, or P;

X2 is any amino acid other than C, preferably L, R, S, D, or Y;

X3 is any amino acid other than C, preferably E, M, or W;

X4 is any amino acid other than C, preferably E, Q, D, F, or S;

X5 is any amino acid other than C, preferably F, I, W, or E;

X6 is any amino acid other than C, preferably D, S, or N;

X7 is any amino acid other than C, preferably D, S, or L;

X8 is any amino acid other than C, preferably D, K, or E;

X9 is any amino acid other than C, preferably T, F, or G;

X10 is any amino acid other than C, preferably F, W, Y, or G;

X11 is any amino acid other than C, preferably E, S, or W;

X12 is any amino acid other than C, preferably E, V, F, or Y;

X13 is any amino acid other than C, preferably T, E, K, or V;

X14 is any amino acid other than C, preferably W or E;

X15 is any amino acid other than C, preferably D, W, F, P, or S; and

X16 is any amino acid other than C, preferably N, I, or A.

CLASS IX

TN9 #3:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:405	606-B08,	SETRPTEAGSCHCSGPPTFQCWCYEVEPTE
SEQ ID NO:406	602-G12,	SETRPTEAGSCHCSGPPTFECWCYGTEPTE
SEQ ID NO:407	603-E09,	SETRPTGESDCHCSGPPTFECYCYGTEPTE
SEQ ID NO:408	606-C12,	SETRPTESGNCYCSGPPWFECWCYGTEPTE
SEQ ID NO:409	603-H03,	SETRPTEAGACRCSGPPTFECYCYDMAPTE
SEQ ID NO:410	604-G01,	SETRPTEAGSCYCSGPPRFECWCYETEPTE
SEQ ID NO:411	602-G04,	SETRPTEAGSCHCSGPPSFECWCFGTEPTE
SEQ ID NO:412	611-G11,	SETRPTVSVSCSCGGPPTFECWCFGTEPTE
SEQ ID NO:413	611-F02,	SETRPTEAGSCHCNGPPTFECFCFGTEPTE
SEQ ID NO:414	610-G02,	SETRPTEAGSCYCGGPPSFECWCYGTEPTE
SEQ ID NO:415	614-E08,	SETRPTEAGSCHCSGPPTFECWCYGSNPTE
SEQ ID NO:416	615-A01,	SETRPTEAGSCHCSGPPAFECWCYRAEPTE
SEQ ID NO:417	617-H02,	SETRPTEAGSCDCSGPPTFECWCFGTEPTE
SEQ ID NO:418	616-F12,	SETRPTEAGKCHCGGPPSFECWCYATEPTE
SEQ ID NO:419	620-G06,	SETRPTEAGKCHCSGPPTFECTCYHTDPTE
SEQ ID NO:420	627-B04,	SETRPTEAGFCQCSGPPAFECWCYDTEPTE
SEQ ID NO:421	627-B06,	SETRPTEAVSCECKGPPTFECWCFGTEPTE
SEQ ID NO:422	626-H05,	SETRPTEAGDCHCSGPPTFECWCYGTEPTE
SEQ ID NO:423	626-D11,	SETRPTEAGACDCIGPPTFECWCYDTYPTE
SEQ ID NO:424	626-E05,	SETRPTEAGNCLCSGPPTFECACYHSEPTE
SEQ ID NO:425	621-D01,	SETRPTEAGSCHCSGPPTFQCWCYSTEPTE
SEQ ID NO:426	622-A10,	SETRPTEAGICHCSGPPTFECWCYATEPTE
SEQ ID NO:427	630-D09,	SETRPTEEGSCHCSGPPTFECWCFGTEPTE
SEQ ID NO:428	628-D01,	SETRPTEAGICNCSGPPTFECWCYSMGPTE
SEQ ID NO:429	628-F11,	SETRPTQGGNCHCSGPPTFECWCYGTEPTE
SEQ ID NO:430	628-D04,	SETRPTEAGSCNCSGPPTFECYCYTLDPTE
SEQ ID NO:431	630-G01,	SETRPTDNGSCQCSGPPTFECWCFGTEPTE
SEQ ID NO:432	627-G06,	SETRPTESGSCHCSGPPTFECWCYGTEPTE
SEQ ID NO:433	630-G05,	SETRPTEAGSCNCSGPPSFECWCYVTEPTE

SEQ ID NO:434 630-C03, SETRPTEGGSCYCGGPPTFECWCYGTEPTE
SEQ ID NO:435 627-G07, SETRPTEAGRCHCSGPPTFECWCYVQEPTE
SEQ ID NO:436 630-H10, SETRPTESGSCLCSGPPQFECWCYGTEPTE
SEQ ID NO:437 628-B01, SETRPTETDSCHCIGPPTFECWCYGTEPTE
SEQ ID NO:438 630-F01, SETRPTEAGFCRCSGPPTFECWCYDTEPTE
SEQ ID NO:439 629-D01, SETRPTEHGSCNCYGPPTFECWCYGTEPTE
SEQ ID NO:440 633-G02, SETRPTALGGCLCSGPPTFECWCYGTEPTE
SEQ ID NO:441 631-F07, SETRPTEGGSCECSGPPTFECWCYGTEPTE
SEQ ID NO:442 633-G08, SETRPTEEGSCHCSGPPAFECWCYGTEPTE
SEQ ID NO:443 632-H07, SETRPTEAGTCYCSGPPTFECWCYGTEPTE
SEQ ID NO:444 631-D03, SETRPTEDGSCHCSGPPRFECWCYGTEPTE
SEQ ID NO:445 633-G12, SETRPTEAGSCHCSGPPTFECWCYSTEPTE
SEQ ID NO:446 633-H03, SETRPTEAGSCYCSGPPTFECWCYAEEPTE
SEQ ID NO:447 632-F05, SETRPTEAGSCHCSGPPTFECWCFEPEPTE

Motif13-1 G-X1-C-X2-C-X3-G-P-P-X4-F-X5-C-X6-C-X7-X8-X9-X10-P, where
X1 is any amino acid other than C, preferably S;
X2 is any amino acid other than C, preferably H, Y, or N;
X3 is any amino acid other than C, preferably S or G;
X4 is any amino acid other than C, preferably T;
X5 is any amino acid other than C, preferably E;
X6 is any amino acid other than C, preferably W;
X7 is any amino acid other than C, preferably Y;
X8 is any amino acid other than C, preferably G, D, A, E, or S;
X9 is any amino acid other than C, preferably T or S; and
X10 is any amino acid other than C, preferably E or D.

Motif13-2 is T-X1-X2-X3-X4-C-X5-C-X6-G-P-P-X7-F-E-C-X8-C-X9-G where:

X1 is any amino acid other than C, preferably E;
X2 is any amino acid other than C, preferably A, S, E, or G;
X3 is any amino acid other than C, preferably G;
X4 is any amino acid other than C, preferably S;
X5 is any amino acid other than C, preferably H;
X6 is any amino acid other than C, preferably S;
X7 is any amino acid other than C, preferably T;
X8 is any amino acid other than C, preferably W, Y, or F; and
X9 is any amino acid other than C, preferably Y or F.

CLASS X

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:448	606-E11,	SEYPTWVSKEFHECAGELVAMQGGSGTE

CLASS XI

Linear #1:

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:449	525-A07,	AQQASRFTFTDGDSYWWFEDF
SEQ ID NO:450	528-F05,	AQIQGIQKTEQGEFYWFNWFPA
SEQ ID NO:451	524-E09,	AQREVEEPYWYLDFLSSWRMHE
SEQ ID NO:452	96-H12 ,	AQRPEAHYKLAMSYPIIPRTKT
SEQ ID NO:453	118-A08,	AQRWSSPGMSQSFVLEWKWNDN
SEQ ID NO:454	94-E08 ,	AQYDTWVFQFIHEVPGELVAMQ
SEQ ID NO:455	119-F06,	AQMYQTPDGVIGKFVDWMFN
SEQ ID NO:456	95-A11 ,	AQVGSPMLPSWFSFEANWSS
SEQ ID NO:457	94-H04 ,	AQNAVVPPPMLWSIYWDYGREG
SEQ ID NO:458	94-F07 ,	AQPYYELQDADMLLVVALLSTG
SEQ ID NO:459	103-G08,	AQVGTAEAIMFSDVEDTGVHKF
SEQ ID NO:460	118-C07,	AQFPLEFDVPNFSYHWLVSFNP
SEQ ID NO:461	104-C09,	AQDLKPWTAGWEPPWLWTDRGP

SEQ ID NO:462	117-F08,	AQHQYGQMMVLHIQYDMGEFIP
SEQ ID NO:463	76-D09 ,	AQSPYIFPIDDSGRQIFVIQWG
SEQ ID NO:464	93-C08 ,	AQVPDWLSAVVIEKLIEYGMMV
SEQ ID NO:465	92-B05 ,	AQFDRYWHFAWMDVSFSSGQSG
SEQ ID NO:466	116-H02,	AQKETWEFFDIVYGSGWKFNSP
SEQ ID NO:467	02-B08 ,	AQHSVQRQMDVWMPVQFMAGFT
SEQ ID NO:468	117-F03,	AQEWQTWTWNMIEVISENKTP
SEQ ID NO:469	127-A07,	AQGFELWVDHTRNFFIAISP
SEQ ID NO:470	94-B08 ,	AQAYEWWADESIFNHGYYWGHQ
SEQ ID NO:471	115-G02,	AQDPGFSKHSMGHGYPSKMNWG
SEQ ID NO:472	130-E10,	AQEWEREYFVDGFWGSWFGIPH
SEQ ID NO:473	136-D01,	AQMGHHWDVQWDYKLFHVARGD
SEQ ID NO:474	15-D02 ,	AQELFQILEKQMWSDFMEWATP
SEQ ID NO:475	79-B02 ,	AQHWDYDSGSDFWFPVFFLEHH
SEQ ID NO:476	94-A06 ,	AQHGYLSPLKQYQMSHVEFWTY
SEQ ID NO:477	94-G02 ,	AQFSGLVMYGRTHEVQWTFGSM
SEQ ID NO:478	75-B12 ,	AQAEWVITSEEFYWKMADFGPP
SEQ ID NO:479	117-F04,	AQWPHDGLVHWGEVIMLRF
SEQ ID NO:480	151-B08,	AQWNQWDEFMWFLNPPPIGLMW
SEQ ID NO:481	117-E09,	AQDNTADQMFNGFHVLAMYMV
SEQ ID NO:482	93-B10 ,	AQSDHDHAHWGVKHWPFRRYQ
SEQ ID NO:483	98-F05 ,	AQLFQYLWHDDPQGAFFQLSMW
SEQ ID NO:484	118-B12,	AQHVVTLTLIQMPFAFNFEPRM
SEQ ID NO:485	27-D10 ,	AQVGESLDDGWTFFSDKWFDFF
SEQ ID NO:486	122-D07,	AQFMYEKEHYVMSISLPGLWFY
SEQ ID NO:487	149-E06,	AQHMDPAEWDWFIRIYSPVVNP
SEQ ID NO:488	166-H04,	AQMWHRVHDPGYTFEVTWLWDN
SEQ ID NO:489	96-D06 ,	AQWNWDMGFMWTTDSAQVQPSM
SEQ ID NO:490	103-C04,	AQKTWFLEADLFQMFQEVTWQF
SEQ ID NO:491	527-E08,	AQWGAVDNDWYDWEMEQIWMFE
SEQ ID NO:492	524-H02,	AQVEDMATVHFKFNPATHEVIW
SEQ ID NO:493	523-A04,	AQRDYLFYWNDGSYQPWQVFVG

SEQ ID NO:494	524-D07,	AQQWMFQIHQSMAWPYEWIDSY
SEQ ID NO:495	522-H03,	AQGIAWQLEWSYMPQSPPSFDR
SEQ ID NO:496	527-A10,	AQGGRYPFYDTDWFKWEMYVL

CLASS XII

Linear #2

SEQ ID NO:	Isolate	Sequence
SEQ ID NO:497	594-F01,	SEEDTWLFWQIIEVPVGQVLMQGGSGTE
SEQ ID NO:498	592-E11,	SEYDTLLFQRTGEVVGKLGSMQGGSGTE
SEQ ID NO:499	591-G09,	SEYDTWVFQFMLEVPGSWMARLGGSGTE
SEQ ID NO:500	601-G11,	SEYDTWIFQFYREVPGVPGAMQGGSGTE
SEQ ID NO:501	592-G01,	SEVDTGVQLLTHEGPGELVAMQGGSGTE
SEQ ID NO:502	591-H01,	SESDTWVFQLIHEVPASVVAMQGGSGTE
SEQ ID NO:503	592-G05,	SEYDTWVFQFRHGVKAQLVAMRGGSGTE
SEQ ID NO:504	606-D12,	SEYDSRVFQYAPEVAGQVEAMQGGSGTE
SEQ ID NO:505	592-B01,	SEDESRVVQFQHEVSGELVAMQGGSGTE
SEQ ID NO:506	591-A06,	SEQDTFVFMYNGEVSGDMVAMQGGSGTE
SEQ ID NO:507	588-H01,	SEYDTWVFQFRRQVPGVLETMLGGSGTE
SEQ ID NO:508	589-A01,	SEQETLVFAVIDGDPGELVAMQGGSGTE
SEQ ID NO:509	619-F10,	SEYDTWVFQFIHVARGEMEGTLGGSGTE
SEQ ID NO:510	592-B01,	SEDESRVVQFQHEVSGELVAMQGGSGTE
SEQ ID NO:511	591-A06,	SEQDTFVFMYNGEVSGDMVAMQGGSGTE

Table 7.

CLASS I SEQ ID NO:	Isolate	Protein ELISA	WC ELISA	HGF 100ng/mL	HGF 500ng/mL
SEQ ID NO:001	571-C05	4.9	1.30	102%	74%
SEQ ID NO:002	465-A06	4.4	1.33	56%	32%
SEQ ID NO:003	465-D09	3.2	1.30	90%	70%
SEQ ID NO:004	569-H10	3.4	1.27	98%	83%
SEQ ID NO:005	470-E11	3.5	1.33	55%	127%
SEQ ID NO:006	452-F01	3.2	1.33	117%	110%
SEQ ID NO:007	569-C03	3.4	1.30	95%	89%
SEQ ID NO:008	574-H03	3.2	1.27	88%	18%
SEQ ID NO:009	567-C08	3.8	1.27	85%	94%
SEQ ID NO:010	561-C08	3.0	1.37	92%	96%
CLASS II SEQ ID NO:	Isolate	Protein ELISA	WC ELISA	HGF 100ng/mL	HGF 500ng/mL
SEQ ID NO:011	573-F04	5.6	1.30	76%	71%
SEQ ID NO:012	570-E07	4.5	1.27	81%	71%
SEQ ID NO:013	456-E04	3.9	1.40	82%	81%
SEQ ID NO:014	434-E12	4.8	1.33	117%	41%
SEQ ID NO:015	489-A04	4.3	1.33	30%	13%
SEQ ID NO:016	484-D08	4.1	1.33	105%	90%
SEQ ID	482-D02	3.9	1.37	66%	44%

NO:017					
SEQ ID NO:018	437-A09	3.9	1.13	89%	78%
SEQ ID NO:019	352-E04	3.9	1.37	88%	74%
SEQ ID NO:020	376-E05	3.7	1.37	122%	121%
SEQ ID NO:021	482-A12	3.5	1.37	98%	79%
SEQ ID NO:022	423-C11	3.4	1.40	132%	75%
SEQ ID NO:023	499-C09	3.2	1.33	91%	70%
SEQ ID NO:024	457-A09	14.5	1.30	27%	67%
SEQ ID NO:025	573-E07	3.2	1.37	77%	82%
SEQ ID NO:026	465-F08	3.8	1.30	68%	116%
SEQ ID NO:027	465-E09	3.6	1.30	60%	77%
SEQ ID NO:028	444-B08	3.6	1.43	111%	93%
SEQ ID NO:029	465-E11	4.3	1.23	33%	124%
SEQ ID NO:030	465-D12	3.2	1.27	34%	0%
SEQ ID NO:031	470-A02	3.2	1.30	78%	62%
SEQ ID NO:032	465-C01	3.2	1.27	267%	23%
SEQ ID NO:033	448-H02	3.8	1.43	113%	92%
SEQ ID NO:034	465-D01	3.3	1.30	235%	134%
SEQ ID NO:035	571-C11	3.5	1.23	107%	72%
SEQ ID NO:036	465-B11	3.6	1.27	97%	89%
SEQ ID NO:037	442-E08	4.1	1.43	81%	75%
SEQ ID NO:038	465-C11	3.1	1.30	41%	4%
SEQ ID	465-F10	3.7	1.33	61%	42%

NO:039					
SEQ ID NO:040	471-A11	3.0	1.37	85%	80%
SEQ ID NO:041	465-C07	3.1	1.27	102%	138%
SEQ ID NO:042	465-D04	3.1	1.23	77%	31%
SEQ ID NO:043	445-E04	4.2	1.37	127%	102%
SEQ ID NO:044	465-B06	4.1	1.23	89%	57%
SEQ ID NO:045	470-C02	3.9	1.33	340%	227%
SEQ ID NO:046	458-B05	4.5	1.33	201%	247%
SEQ ID NO:047	545-E08	4.7	1.30	81%	57%

CLASS III

SEQ ID NO:	Isolate	Protein ELISA	WC ELISA	HGF 100ng/mL	HGF 500ng/mL
SEQ ID NO:048	325-H05	15.9	1.47	41%	32%
SEQ ID NO:049	330-F05	13.8	1.33	51%	27%
SEQ ID NO:050	333-F09	14.8	1.43	52%	32%
SEQ ID NO:051	336-G04	5.4	1.33	46%	23%
SEQ ID NO:052	334-G06	8.0	1.30	56%	43%
SEQ ID NO:053	330-B07	18.1	1.27	58%	40%
SEQ ID NO:054	330-C10	13.4	1.33	48%	25%
SEQ ID NO:055	331-G04	18.3	1.47	56%	36%
SEQ ID NO:056	548-F06	14.3	1.23	76%	18%
SEQ ID NO:057	538-F08	12.3	1.23	55%	43%
SEQ ID NO:058	547-H07	15.9	1.17	60%	45%
SEQ ID	323-A11	21.2	1.43	41%	18%

NO:059					
SEQ ID NO:060	333-H03	8.1	1.43	55%	37%
SEQ ID NO:061	329-D02	3.2	1.27	53%	31%
SEQ ID NO:062	550-C09	10.2	1.40	25%	25%
SEQ ID NO:063	548-E08	5.3	1.27	102%	50%
SEQ ID NO:064	332-A05	6.0	1.40	40%	21%
SEQ ID NO:065	330-C01	4.7	1.30	58%	43%
SEQ ID NO:066	545-A09	13.5	1.30	44%	22%
SEQ ID NO:067	334-C08	8.0	1.47	70%	57%
SEQ ID NO:068	333-C05	6.3	1.33	83%	66%
SEQ ID NO:069	551-B02	9.0	1.30	69%	43%
SEQ ID NO:070	551-G12	3.9	1.37	88%	46%
SEQ ID NO:071	330-G09	13.5	1.40	42%	26%
SEQ ID NO:072	331-F01	12.6	1.47	77%	73%
SEQ ID NO:073	274-B07	7.8	1.10	342%	296%
SEQ ID NO:074	335-D11	6.7	1.37	56%	37%
SEQ ID NO:075	336-D07	5.8	1.33	44%	37%
SEQ ID NO:076	332-C03	5.7	1.20	37%	95%
SEQ ID NO:077	331-D03	5.5	1.40	64%	55%
SEQ ID NO:078	331-G06	4.7	1.40	59%	51%
SEQ ID NO:079	552-G03	10.7	1.27	101%	83%
SEQ ID NO:080	552-G11	7.4	1.23	55%	41%
SEQ ID	550-G08	9.1	1.40	79%	58%

NO:081					
SEQ ID NO:082	550-G12	14.3	1.43	61%	79%
SEQ ID NO:083	552-A01	3.9	1.33	76%	81%
SEQ ID NO:084	548-C06	13.0	1.23	94%	77%
SEQ ID NO:085	545-B12	17.1	1.27	51%	42%
SEQ ID NO:086	549-F06	5.2	1.30	96%	40%
SEQ ID NO:087	552-F01	4.8	1.30	56%	37%
SEQ ID NO:088	547-H12	5.6	1.10	92%	81%
SEQ ID NO:089	550-F11	12.4	1.23	58%	23%
SEQ ID NO:090	548-D08	19.5	1.23	97%	62%
SEQ ID NO:091	549-D02	8.9	1.27	47%	36%
SEQ ID NO:092	552-F02	12.3	1.23	60%	40%
SEQ ID NO:093	545-E04	16.3	1.23	48%	17%
SEQ ID NO:094	545-E05	10.3	1.27	70%	32%
SEQ ID NO:095	547-H03	16.2	1.23	109%	53%
SEQ ID NO:096	552-G09	9.7	1.27	98%	68%
SEQ ID NO:097	550-A08	8.4	1.27	52%	51%
SEQ ID NO:098	550-G07	6.2	1.27	63%	36%
SEQ ID NO:099	551-A05	4.0	1.30	68%	42%
SEQ ID NO:100	548-C10	8.4	1.20	69%	57%
SEQ ID NO:101	465-C10	3.0	1.27	95%	71%
CLASS V					
SEQ ID NO:	Isolate	Protein ELISA	WC ELISA	HGF 100ng/m	HGF 500ng/mL

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SEQ ID NO:365	545-C02	26.3	1.33	54%	31%
SEQ ID NO:366	546-E02	10.4	1.33	74%	54%
SEQ ID NO:367	545-C11	7.7	1.30	77%	50%
SEQ ID NO:368	549-G01	7.0	1.27	62%	18%
SEQ ID NO:369	548-F07	27.5	2.43	54%	37%
SEQ ID NO:370	551-H10	13.3	1.87	88%	49%
CLASS VI					
SEQ ID NO:	Isolate	Protein ELISA	WC ELISA	HGF 100ng/mL	HGF 500ng/mL
SEQ ID NO:371	443-H10	3.4	1.40	124%	143%
SEQ ID NO:372	557-A12	4.6	1.37	87%	62%
SEQ ID NO:373	465-A03	4.0	1.30	33%	17%
SEQ ID NO:374	446-E12	3.3	1.37	73%	83%
SEQ ID NO:375	445-E06	4.3	1.33	83%	73%
SEQ ID NO:376	452-A03	3.0	1.30	140%	112%
SEQ ID NO:377	465-C06	6.4	1.23	184%	104%
SEQ ID NO:378	441-H01	3.6	1.40	91%	69%
SEQ ID NO:379	443-D04	3.2	1.43	69%	73%
SEQ ID NO:380	445-G12	4.0	1.37	85%	52%
SEQ ID NO:381	442-E03	3.9	1.43	130%	81%
SEQ ID NO:382	453-A05	4.5	1.33	51%	28%
SEQ ID NO:383	445-E07	3.1	1.37	82%	64%
SEQ ID	451-B12	3.1	1.37	61%	27%

NO:384					
SEQ ID NO:385	465-B07	4.8	1.27	111%	79%
SEQ ID NO:386	451-C06	3.0	1.37	108%	86%
SEQ ID NO:387	445-E11	3.7	1.43	69%	79%
CLASS VIII					
SEQ ID NO:	Isolate	Protein ELISA	WC ELISA	HGF 100ng/mL	HGF 500ng/mL
SEQ ID NO:390	546-G02	16.1	1.27	32%	19%
SEQ ID NO:391	333-C03	12.4	1.37	52%	43%
SEQ ID NO:392	549-G05	23.7	1.47	28%	21%
SEQ ID NO:393	546-B01	8.4	1.20	95%	77%
SEQ ID NO:394	551-D02	13.4	1.37	91%	70%
SEQ ID NO:395	334-F05	13.5	1.40	58%	29%
SEQ ID NO:396	330-G02	7.4	1.30	37%	31%
SEQ ID NO:397	316-F08	7.0	1.30	72%	38%
SEQ ID NO:398	332-H09	6.2	1.30	50%	43%
SEQ ID NO:399	545-H12	11.3	1.30	74%	60%
SEQ ID NO:400	548-G05	6.1	1.30	110%	47%
SEQ ID NO:401	547-C09	4.3	1.23	50%	32%
SEQ ID NO:402	545-F04	5.2	1.17	143%	114%
SEQ ID NO:403	552-F06	11.1	1.23	82%	32%
SEQ ID NO:404	531-E11	3.4	1.30	61%	33%
CLASS XI					
SEQ ID NO:	Isolate	Protein ELISA	WC ELISA	HGF 100ng/m	HGF 500ng/mL

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SEQ ID NO:449	525-A07	7.0	1.17	93%	88%
SEQ ID NO:450	528-F05	4.3	1.10	84%	81%
SEQ ID NO:451	524-E09	8.2	1.33	100%	93%
SEQ ID NO:452	96-H12	35.3	1.37	88%	64%
SEQ ID NO:453	118-A08	11.3	1.30	85%	74%
SEQ ID NO:454	94-E08	8.9	1.23	102%	74%
SEQ ID NO:455	119-F06	8.0	1.33	4%	27%
SEQ ID NO:456	95-A11	7.0	1.30	109%	108%
SEQ ID NO:457	94-H04	7.0	1.37	150%	101%
SEQ ID NO:458	94-F07	6.1	1.20	106%	104%
SEQ ID NO:459	103-G08	5.7	1.33	140%	95%
SEQ ID NO:460	118-C07	5.6	1.27	100%	84%
SEQ ID NO:461	104-C09	5.0	1.30	64%	50%
SEQ ID NO:462	117-F08	4.5	1.27	102%	270%
SEQ ID NO:463	76-D09	4.4	1.23	79%	87%
SEQ ID NO:464	93-C08	4.4	1.37	101%	96%
SEQ ID NO:465	92-B05	4.3	1.20	94%	94%
SEQ ID NO:466	116-H02	4.0	1.23	84%	72%
SEQ ID NO:467	02-B08	3.9	1.30	84%	96%
SEQ ID NO:468	117-F03	3.8	1.40	104%	93%
SEQ ID NO:469	127-A07	3.8	1.20	101%	107%
SEQ ID	94-B08	3.8	1.20	111%	121%

NO:470					
SEQ ID NO:471	115-G02	3.7	1.27	59%	0%
SEQ ID NO:472	130-E10	3.7	1.80	100%	92%
SEQ ID NO:473	136-D01	3.7	1.23	85%	149%
SEQ ID NO:474	15-D02	3.6	1.23	97%	118%
SEQ ID NO:475	79-B02	3.5	1.30	102%	86%
SEQ ID NO:476	94-A06	3.5	1.17	84%	96%
SEQ ID NO:477	94-G02	3.5	1.30	108%	76%
SEQ ID NO:478	75-B12	3.4	1.23	95%	108%
SEQ ID NO:479	117-F04	3.3	1.37	93%	91%
SEQ ID NO:480	151-B08	3.3	1.23	102%	368%
SEQ ID NO:481	117-E09	3.3	1.37	109%	102%
SEQ ID NO:482	93-B10	3.1	1.20	0%	0%
SEQ ID NO:483	98-F05	3.1	1.23	88%	57%
SEQ ID NO:484	118-B12	3.1	1.30	98%	112%
SEQ ID NO:485	27-D10	3.0	1.17	111%	131%
SEQ ID NO:486	122-D07	3.0	1.63	102%	92%
SEQ ID NO:487	149-E06	3.0	1.80	80%	86%
SEQ ID NO:488	166-H04	3.0	1.27	77%	85%
SEQ ID NO:489	96-D06	3.0	1.37	154%	151%
SEQ ID NO:490	103-C04	3.0	1.40	73%	86%
SEQ ID NO:491	527-E08	3.2	1.23	98%	95%
SEQ ID	524-H02	3.2	1.53	26%	25%

NO:492					
SEQ ID NO:493	523-A04	5.5	1.30	133%	143%
SEQ ID NO:494	524-D07	3.9	1.23	105%	104%
SEQ ID NO:495	522-H03	4.5	1.17	107%	94%
SEQ ID NO:496	527-A10	3.8	1.30	84%	78%

Note: Protein ELISAs were measured as fold over background (cMet-Fc vs. TRAIL-Fc)
Whole Cell ELISAs were measured as fold over background (3T3 cells expressing human cMet vs. non-expressing 3T3 cells)
HGF competition ELISA measured as a % of binding in the absence of HGF.

Table 8: Fluorescence polarization analysis of select peptides from first generation peptide library positive hits

CLASS I			
SEQ ID NO:	Isolate	Kd (human)	Kd (mouse)
SEQ ID NO:001	571-C05	0.20	3.50
CLASS III			
SEQ ID NO:	Isolate	Kd (human)	Kd (mouse)
SEQ ID NO:048	325-H05	3.50	NT
SEQ ID NO:051	336-G04	3.20	NT
SEQ ID NO:052	334-G06	2.70	NT
SEQ ID NO:053	330-B07	2.90	NT
SEQ ID NO:055	331-G04	0.90	1.10
SEQ ID NO:056	548-F06	2.70	NT
SEQ ID NO:059	323-A11	4.30	NT
SEQ ID NO:061	329-D02	5.20	NT
SEQ ID NO:067	334-C08	1.65	NT
SEQ ID NO:068	333-C05	2.80	NT
SEQ ID NO:071	330-G09	1.85	NT
SEQ ID NO:072	331-F01	0.98	NT
SEQ ID NO:074	335-D11	3.30	NT
SEQ ID NO:078	331-G06	2.90	NT
CLASS V			
SEQ ID NO:	Isolate	Kd (human)	Kd (mouse)
SEQ ID NO:369	548-F07	0.88	NB
SEQ ID NO:370	551-H10	0.22	NB

CLASS VIII			
SEQ ID NO:	Isolate	Kd (human)	Kd (mouse)
SEQ ID NO:390	546-G02	1.50	NT
SEQ ID NO:391	333-C03	1.80	NT
SEQ ID NO:399	545-H12	1.15	NB
CLASS XI			
SEQ ID NO:	Isolate	Kd (human)	Kd (mouse)
SEQ ID NO:449	525-A07	6.90	NT
SEQ ID NO:450	528-F05	2.70	NT
SEQ ID NO:451	524-E09	2.00	NT
SEQ ID NO:452	96-H12	>2.00	NT
SEQ ID NO:453	118-A08	>2.00	NT
SEQ ID NO:454	94-E08	0.93	NT
SEQ ID NO:456	95-A11	2.30	NT
SEQ ID NO:458	94-F07	3.75	NT
SEQ ID NO:459	103-G08	>2.00	NT
SEQ ID NO:461	104-C09	>2.00	NT
SEQ ID NO:462	117-F08	>2.00	NT
SEQ ID NO:463	76-D09	>2.00	NT
SEQ ID NO:464	93-C08	>2.00	NT
SEQ ID NO:466	116-H02	>2.00	NT
SEQ ID NO:467	02-B08	>2.00	NT
SEQ ID NO:469	127-A07	2.40	NT
SEQ ID NO:472	130-E10	2.60	7.65
SEQ ID NO:475	79-B02	1.90	NT
SEQ ID NO:479	117-F04	1.70	NT
SEQ ID NO:492	524-H02	0.80	NT

Kd values are in μM. NB = no binding, NT = not tested

Table 9: cMet-binding heteromeric peptide complexes

PAIR I		
SEQ ID NO:	Isolate	CLASS
SEQ ID NO:472	130-E10	XI
SEQ ID NO:370	551-H10	V
PAIR II		
SEQ ID NO:	Isolate	CLASS
SEQ ID NO:369	548-F07	V
SEQ ID NO:370	551-H10	V

PAIR III		
SEQ ID NO:	Isolate	CLASS
SEQ ID NO:370	551-H10	V
SEQ ID NO:399	545-H12	VIII

Table 10: Amino-acid sequence of Mature HSA from GenBank entry AAN17825

DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKQEPERNE
CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
LECADDRADL AKYICENQDS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
KTYKTTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPCFSA LEVDETYVPK
EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
FAAFVEKCCK ADDKETCFAE EGKKLVAASR AALGL (SEQ ID NO:647)

Table 11: Amino-acid Sequence of SEQ ID NO:648::HSA::SEQ ID NO:649

GSFFPCWRIDRFGYCHANAP GSGGSGG
DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKQEPERNE
CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
LECADDRADL AKYICENQDS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
KTYKTTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPCFSA LEVDETYVPK
EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD

FAAFVEKCCK ADDKETCFAE EGKKLVAASR AALGL
GSGGEGGSG GSWIICWWDNCGSSAP(SEQ ID NO:650)

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A polypeptide or multimeric peptide construct which binds cMet or a complex comprising cMet and HGF.

2. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7-Cys-X_8-X_9-X_{10}, wherein

X_1 is Phe, Leu, Ser, Trp, Tyr or Met;

X_2 is Ile, Tyr, His, Thr or Asn;

X_3 is Ile, Leu, Asp, Met, Phe or Ser;

X_4 is Arg, Asn, Glu, Pro or Trp;

X_5 is Glu, Gly, Leu, Pro, Thr, Trp or Tyr;

X_6 is Asp, Gln, Glu, Gly, Phe, Ser, Thr, or Trp;

X_7 is Ala, Arg, Asn, Gln, Glu, Gly, Phe or Trp;

X_8 is Gly, Asn, His, Arg, Met, Ile, Asp, Val, or Thr;

X_9 is Ser, Lys, Phe, Met, Thr, Asp or Leu; and

X_{10} is Ser, Pro, Thr, Leu, Tyr, Asn, His, Glu or Trp.

3. A polypeptide or multimeric polypeptide according to claim 2, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:9 AND SEQ ID NO:10.

4. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7- X_8-X_9-Cys-X_{10}-X_{11}-X_{12}, wherein

X_1 is Gly, Val, Trp, Thr, Lys or Gln;

X_2 is Trp, Tyr, Leu, Phe or Thr;

X_3 is Trp, Glu, Phe, Ile, Leu or Ser;

X_4 is Asn, Gln or Glu;

X_5 is Leu, Glu or Trp;

X_6 is Glu, Ser or Tyr;

X_7 is Glu, Met or Pro;

X_8 is Met, Ser or Trp;

X_9 is Leu, Phe or Val;

X_{10} is Asp, Glu or Trp;

X_{11} is Met, Phe or Trp; and

X_{12} Gln, Leu, or Trp.

5. A polypeptide or multimeric polypeptide according to claim 4, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:11; SEQ D NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO: 16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; and SEQ ID NO:40.

6. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-Cys-X_4-Gly-X_5-Pro-X_6-Phe-X_7-Cys-X_8-X_9, wherein

X_1 is Glu, Ser, or Trp;

X_2 is Phe-Thr or Trp;

X_3 is His, Phe or Trp;

X_4 is Ala, Lys, Ser or Thr;

X_5 is Pro or Trp;

X_6 is Ser or Thr;

X_7 is Glu or Ser;

X_8 is Ile, Trp or Tyr; and

X_9 is Glu, Met or Tyr.

7. A polypeptide or multimeric polypeptide according to claim 6, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:48; SEQ D

NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID NO:57; SEQ ID NO:58; SEQ ID NO:59; SEQ ID NO:60; SEQ ID NO:61; SEQ ID NO:62; SEQ ID NO:63; SEQ ID NO:64; SEQ ID NO:65; SEQ ID NO:66; SEQ ID NO:67; SEQ ID NO:68; SEQ ID NO:69; SEQ ID NO:70; SEQ ID NO:71; SEQ ID NO:72; SEQ ID NO:73; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:79; SEQ ID NO:80; SEQ ID NO:81; SEQ ID NO:82; SEQ ID NO:83; SEQ ID NO:84; SEQ ID NO:85; SEQ ID NO:86; SEQ ID NO:87; SEQ ID NO:88; SEQ ID NO:89; SEQ ID NO:90; SEQ ID NO:91; SEQ ID NO:92; SEQ ID NO:93; SEQ ID NO:94; SEQ ID NO:95; SEQ ID NO:96; SEQ ID NO:97; SEQ ID NO:98; SEQ ID NO:99; SEQ ID NO:100; and SEQ ID NO:101.

8. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-Cys-X_4-Gly-Pro-Pro-X_5-Phe-X_6-Cys-Trp-X_7-X_8-X_9-X_{10}-X_{11}, wherein

X_1 is Arg, Asp, Asn, Ile or Ser;

X_2 is Leu, Ile, Phe, Trp or Val;

X_3 is Asn, Gln, His, Leu, Tyr or Val;

X_4 is Leu, Lys or Ser;

X_5 is Ala, Ser, Thr or Trp;

X_6 is Glu or Ser;

X_7 is Leu, Ser or Trp;

X_8 is Phe or Tyr;

X_9 is Asp, Glu, Gly or Val;

X_{10} is Met, Pro, Thr or Ser; and

X_{11} is Glu or Gly.

9. A polypeptide or multimeric polypeptide according to claim 8, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:103; SEQ D NO:104; SEQ ID NO:106; SEQ ID NO:108; SEQ ID NO:111; SEQ ID NO:114; SEQ ID NO:117; SEQ ID NO:119; SEQ ID NO:120; SEQ ID NO:121; SEQ ID NO:128; SEQ ID

NO:130; SEQ ID NO:132; SEQ ID NO:133; SEQ ID NO:140; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:146; SEQ ID NO:147; SEQ ID NO:150; SEQ ID NO:151; SEQ ID NO:153; SEQ ID NO:155-SEQ ID NO:158; SEQ ID NO:163; SEQ ID NO:165; SEQ ID NO:167; SEQ ID NO:169; SEQ ID NO:170; SEQ ID NO:172; SEQ ID NO:173; SEQ ID NO:178; SEQ ID NO:179; SEQ ID NO:184; SEQ ID NO:185; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:190; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:198; SEQ ID NO:201- SEQ ID NO:204; SEQ ID NO:208; SEQ ID NO:209; SEQ ID NO:211; SEQ ID NO:215; SEQ ID NO:216; SEQ ID NO:217; SEQ ID NO:219; SEQ ID NO:221; SEQ ID NO:224; SEQ ID NO:226; SEQ ID NO:227; SEQ ID NO:230-SEQ ID NO:234; SEQ ID NO:236; SEQ ID NO:237; SEQ ID NO:238; SEQ ID NO:242; SEQ ID NO:246-SEQ ID NO:254; SEQ ID NO:256; SEQ ID NO:259-SEQ ID NO:264; SEQ ID NO:266; SEQ ID NO:267; SEQ ID NO:269-SEQ ID NO:271; SEQ ID NO:273; SEQ ID NO:275-SEQ ID NO:282; SEQ ID NO:284; SEQ ID NO:285; SEQ ID NO:287; SEQ ID NO:288; SEQ ID NO:293-SEQ ID NO:295; SEQ ID NO:297; SEQ ID NO:298; SEQ ID NO:301-SEQ ID NO:303; SEQ ID NO:305-SEQ ID NO:308; SEQ ID NO:311; SEQ ID NO:313-SEQ ID NO:320; SEQ ID NO:323; SEQ ID NO:324; SEQ ID NO:326-SEQ ID NO:328; SEQ ID NO:331-SEQ ID NO:335; SEQ ID NO:337-SEQ ID NO:341; SEQ ID NO:345; SEQ ID NO:347; SEQ ID NO:349; SEQ ID NO:352; SEQ ID NO:354-SEQ ID NO:357; and SEQ ID NO:361-SEQ ID NO:364.

10. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-X_4-Trp-X_5-Cys-X_6-Gly-Pro-Pro-Thr-Phe-Glu-Cys-Trp-X_7-X_8, wherein

X_1 is Asp, Glu or Val;

X_2 is Ala, Asp, Gly, Ser or Val;

X_3 is Asp, Gly, Ser or Val;

X_4 is Arg, Asn, Gly, Ser or Thr;

X_5 is Gln or His;

X_6 is Asn, Lys or Ser; and

X_7 is Ser or Trp.

11. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7- X_8-X_9-X_{10}-X_{11}-Cys-X_{12}-X_{13}-X_{14}, wherein

X_1 is His, Phe, Pro, Thr or Trp;

X_2 is Ala, Arg, Glu, His, Lys or Phe;

X_3 is Met, Phe, Pro, Thr or Val;

X_4 is His, Leu, Met, Phe or Trp;

X_5 is Arg, Asp, Glu, Gly, Met or Trp;

X_6 is Glu, Gly, Ile, Lys, Phe or Pro;

X_7 is Asp, Phe, Pro, Ser, Trp or Tyr;

X_8 is Ala, Arg, Asn, Phe or Ser;

X_9 is Ala, Gln, Gly, Leu or Phe;

X_{10} is Gln, Gly, Ile, Leu, Trp or Tyr;

X_{11} is Arg, Asp, Phe, Pro, Tyr or Val;

X_{12} is Asn, Gln, His, Ile or Thr;

X_{13} is Ala, Asn, Asp, Glu or His; and

X_{14} is Asn, Gln, Glu, His or Val.

12. A polypeptide or multimeric polypeptide according to claim 11, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:365; SEQ ID NO:366; SEQ ID NO:367; SEQ ID NO:368; SEQ ID NO:369; and SEQ ID NO:370.

13. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7- X_8-X_9-X_{10}-X_{11}- X_{12}-Cys-X_{13}-X_{14}-X_{15}, wherein

X_1 is Gln, Gly, Met, Phe or Ser;

X_2 is Asn, Gln, Leu or Met;

X_3 is Arg, Asn, Gly, His or Ile;

X_4 is Asn, Asp, Leu, Thr or Trp;

X_5 is Arg, Gln, Thr, Tyr or Val;

X_6 is Glu, Gly, Leu, Met or Thr;

X_7 is Ala, Asn, Asp, His, Ile, Leu, or Ser;

X_8 is Arg, Gln, Ser, Thr or Tyr;

X_9 is Asp, Gly, Ile or Phe;

X_{10} is Gln, Phe or Thr;

X_{11} is Gln, His, Phe, Pro, Ser or Tyr;

X_{12} is Asn, Asp, Phe, Pro or Ser;

X_{13} is Ala, Asn, Gly, Leu or Ser;

X_{14} is Arg, Pro, Ser or Val; and

X_{15} is Asp, Glu, Leu or Met.

14. A polypeptide or multimeric polypeptide according to claim 13, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:371; SEQ ID NO:372; SEQ ID NO:373; SEQ ID NO:374; SEQ ID NO:375; SEQ ID NO:376; SEQ ID NO:377; SEQ ID NO:378; SEQ ID NO:379; SEQ ID NO:380; SEQ ID NO:381; SEQ ID NO:382; SEQ ID NO:383; SEQ ID NO:384; SEQ ID NO:385; SEQ ID NO:386; and SEQ ID NO:387.

15. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: X_1-X_2-X_3-Cys-X_4-X_5-X_6-X_7- X_8-X_9-X_{10}-X_{11}- X_{12}- X_{13}-Cys-X_{14}-X_{15}-X_{16}, wherein

X_1 is Ala, His, Leu, Pro or Tyr;

X_2 is Arg, Asp, Leu, Ser or Tyr;

X_3 is Glu, Met or Trp;

X_4 is Asp, Gln, Glu, Phe or Ser;

X_5 is Glu, Ile, Phe or Trp;

X_6 is Asn, Asp or Ser;

X_7 is Asn, Asp or Leu;

X_8 is Asp, Glu or Lys;

X_9 is Gly, Phe or Thr;

X_{10} is Gly, Phe, Trp or Tyr;

X_{11} is Glu, Ser or Trp;

X_{12} is Glu, Phe, Tyr or Val;

X_{13} is Glu, Lys, Thr or Val;

X_{14} is Glu or Trp;

X_{15} is Asp, Phe, Pro, Ser or Trp; and

X_{16} is Ala, Asn or Ile.

16. A polypeptide or multimeric polypeptide according to claim 15, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:390; SEQ ID NO:391; SEQ ID NO:392; SEQ ID NO:393; SEQ ID NO:394; SEQ ID NO:395; SEQ ID NO:396; SEQ ID NO:397; SEQ ID NO:398; SEQ ID NO:399; SEQ ID NO:400; SEQ ID NO:401; SEQ ID NO:402; SEQ ID NO:403; and SEQ ID NO:404.

17. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: Ser-Cys-X_1-Cys-X_2-Gly-Pro-Pro-Thr-Phe-Glu-Cys-Trp-Cys-Tyr-X_3-X_4-X_5, wherein

X_1 is Asn, His or Tyr;

X_2 is Gly or Ser;

X_3 is Ala, Asp, Glu, Gly or Ser;

X_4 is Ser or Thr; and

X_5 is Asp or Glu.

18. A polypeptide or multimeric polypeptide according to claim 17, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:405; SEQ ID NO:406; SEQ ID NO:407; SEQ ID NO:408; SEQ ID NO:409; SEQ ID NO:410; SEQ ID NO:411; SEQ ID NO:412; SEQ ID NO:413; SEQ ID NO:414; SEQ ID NO:415; SEQ ID NO:416; SEQ ID NO:417; SEQ ID NO:418; SEQ ID NO:419; SEQ ID NO:420; SEQ ID NO:421; SEQ ID NO:422; SEQ ID NO:423; SEQ ID NO:424; SEQ ID NO:425; SEQ ID NO:426; SEQ ID NO:427; SEQ ID NO:428; SEQ ID NO:429; SEQ ID NO:430; SEQ ID NO:431; SEQ ID NO:432; SEQ ID NO:433; SEQ ID NO:434; SEQ ID NO:435; SEQ ID NO:436; SEQ ID NO:437; SEQ ID NO:438; SEQ ID NO:439; SEQ ID NO:440; SEQ ID

NO:441; SEQ ID NO:442; SEQ ID NO:443; SEQ ID NO:444; SEQ ID NO:445; SEQ ID NO:446; and SEQ ID NO:447.

19. A polypeptide or multimeric polypeptide according to claim 1, comprising an amino acid sequence: Glu- X_1-Gly-Ser-Cys-His-Cys-Ser-Gly-Pro-Pro-Thr-Phe-Glu-Cys-X_2-Cys-X_3, wherein

X_1 is Ala, Glu, Gly or Ser;

X_2 is Phe, Trp or Tyr; and

X_3 is Phe or Tyr.

20. A polypeptide or multimeric polypeptide according to claim 19, comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:406; SEQ ID NO:415; SEQ ID NO:427; SEQ ID NO:445 and SEQ ID NO:447.

21. A polypeptide or multimeric polypeptide according to any one of claims 2-20, wherein said polypeptide or multimeric polypeptide has a K_d for cMet of less than about 1.0 μM.

22. A multimeric polypeptide according to claim 12, further comprising an amino acid sequence of SEQ ID NO:516 or SEQ ID NO:517.

23. A multimeric polypeptide according to claim 22, wherein the multimeric polypeptide comprises an amino acid sequence of SEQ ID NO:514 or SEQ ID NO:515.

24. A multimeric polypeptide according to claim 12, wherein the multimeric polypeptide comprises SEQ ID NO:514 and SEQ ID NO:515.

25. A method of detecting cMet or a complex of cMet and HGF in an animal or human subject comprising:

providing a polypeptide or multimeric polypeptide according to any of claims 1-24, wherein the polypeptide or multimeric polypeptide is labeled;

administering to the subject the labeled polypeptide or multimeric polypeptide; and

detecting the labeled polypeptide or multimeric polypeptide in the subject.

26. A method according claim 25, wherein the label is radioactive or paramagnetic.

27. A method of treating a condition involving activation of cMet, comprising:

administering to an animal or human subject a composition comprising a polypeptide or multimeric polypeptide according to any of claims 1-24.

28. A method according to claim 27, wherein the disease is a solid tumor.

29. A method according to claim 28, wherein the tumor is selected from the group consisting of: breast, thyroid, glioblastoma, prostate, malignant mesothelioma, colorectal, heptacellular, heptobiliary, renal, osteosarcoma and cervical tumors.

30. A method of purifying cMet or a cMet and HGF complex from a solution containing it, comprising:

contacting the solution with at least one polypeptide or multimeric polypeptide according to any of claims 1-24; and

separating the polypeptide or multimeric polypeptide from the solution.

31. A recombinant bacteriophage expressing exogenous DNA encoding a polypeptide of any of claims 1-21, wherein the polypeptide is displayed on the surface of the bacteriophage.

FIG. 1A

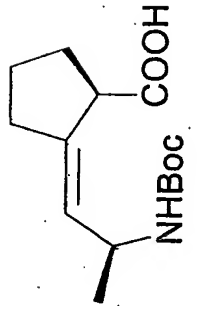


FIG. 1B

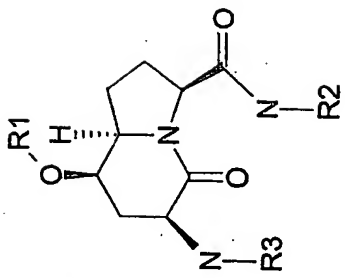


FIG. 1C

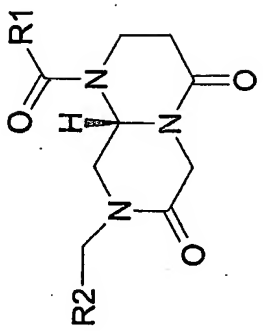
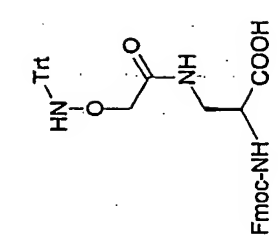
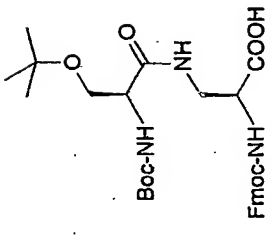


FIG. 2



Fmoc-Dap(Trt-Aoa)-OH

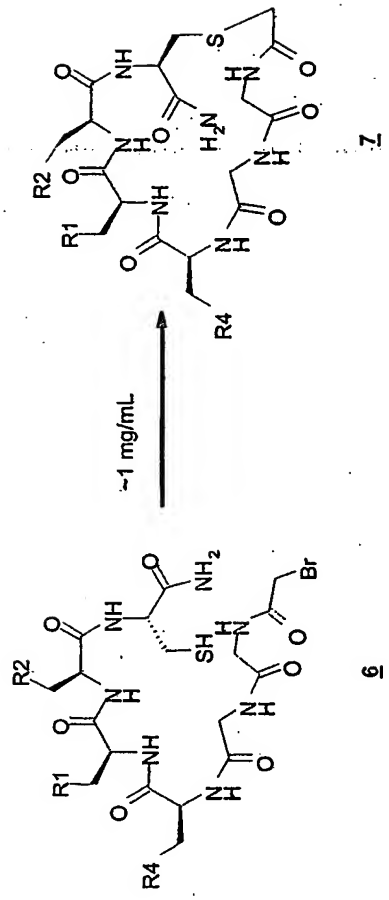
5



Fmoc-Dap(Boc-Ser(t-Bu))-OH

4

FIG. 3



7

6

FIG. 4

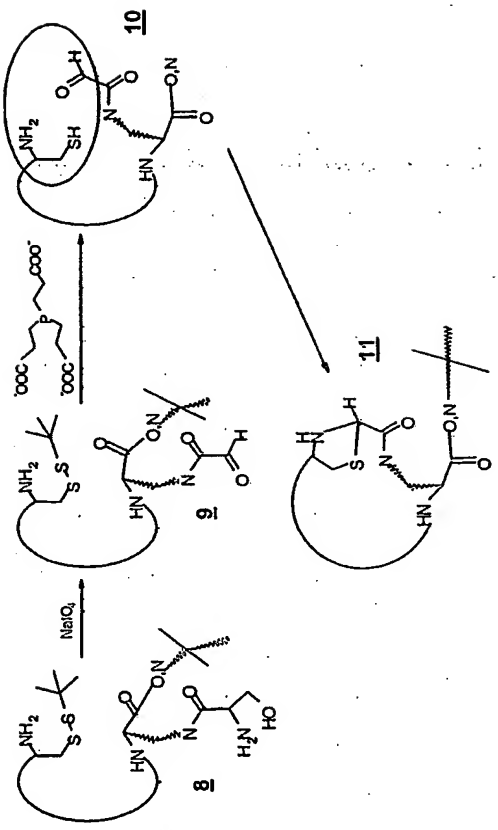


FIG. 5

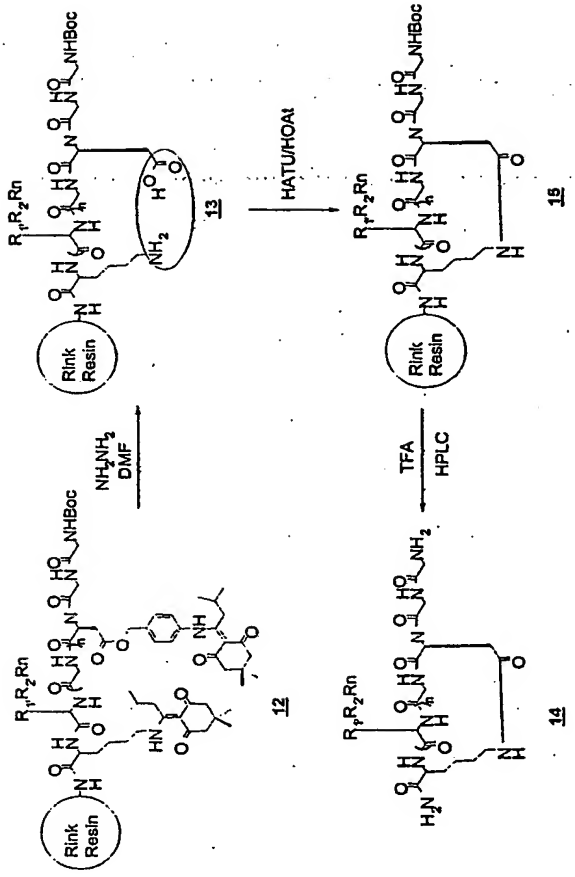


FIG. 6

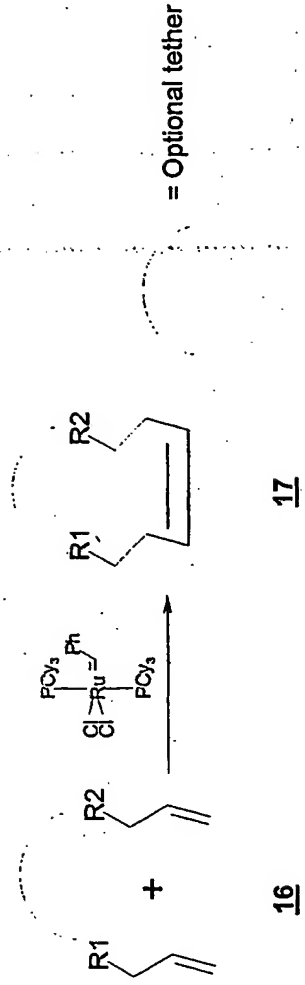


FIG. 7A

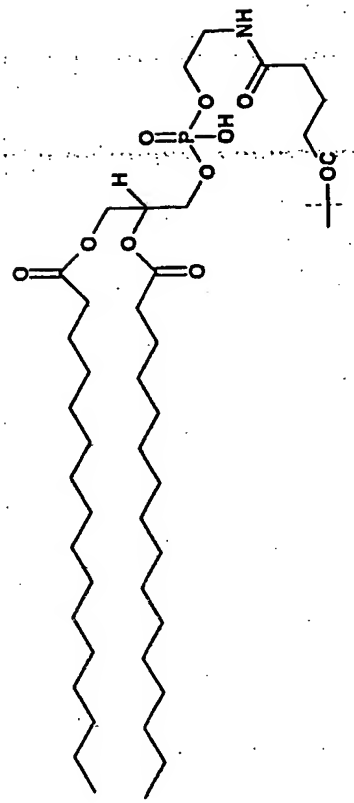


FIG. 7B

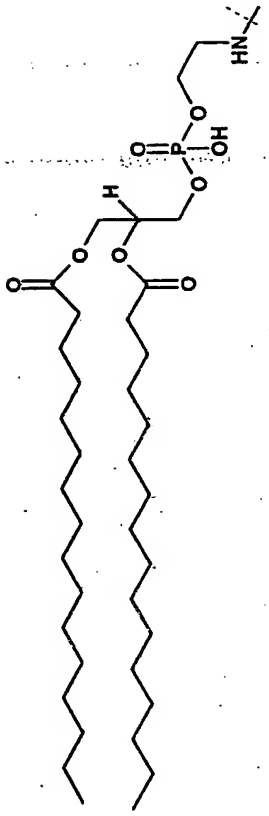


FIG. 8A

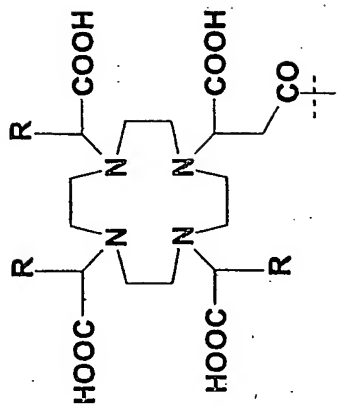


FIG. 8B

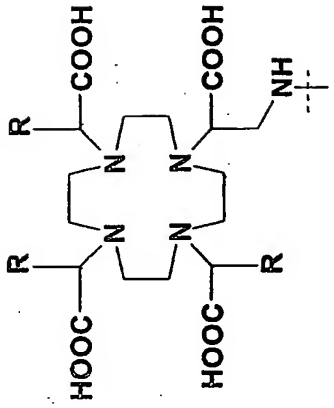


FIG. 8C

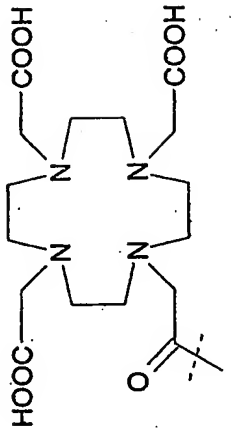


FIG. 8D

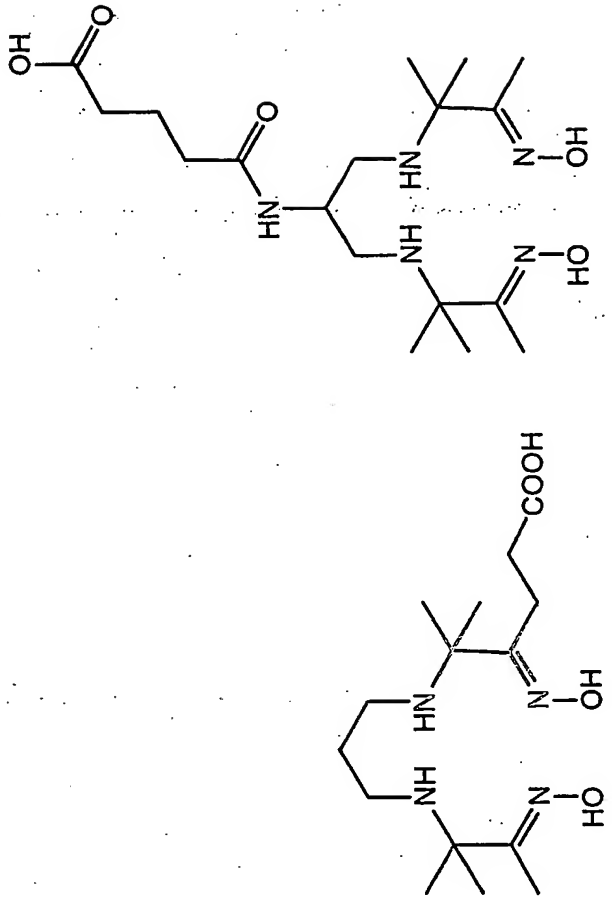


FIG. 8E

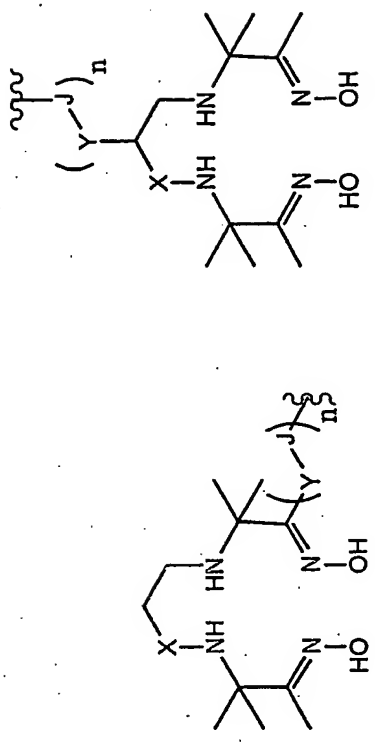


FIG. 8F

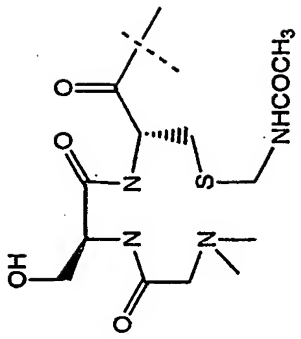


FIG. 9

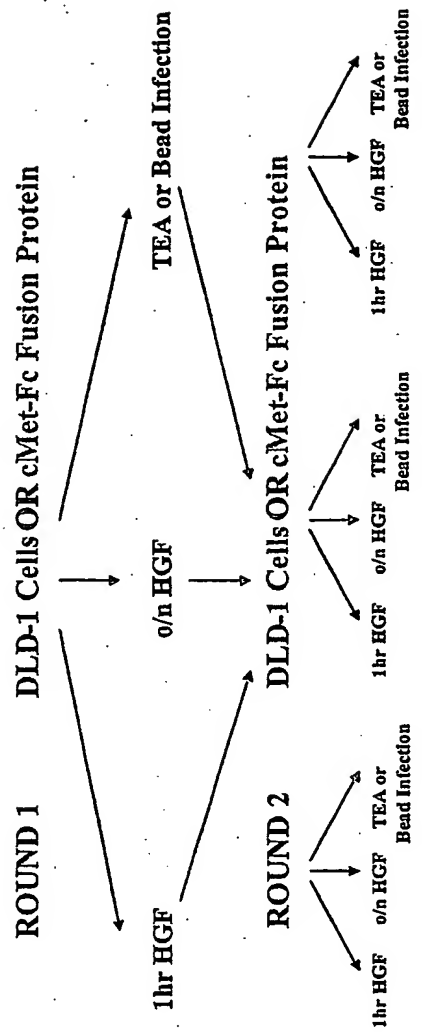

ROUND 1
DLD-1 Cells OR cMet-Fc Fusion Protein
TEA or Bead Infection
o/n HGF
1hr HGF
ROUND 2
DLD-1 Cells OR cMet-Fc Fusion Protein
1hr HGF
o/n HGF
TEA or Bead Infection
1hr HGF
o/n HGF
TEA or Bead Infection
1hr HGF
o/n HGF
TEA or Bead Infection

FIG. 10

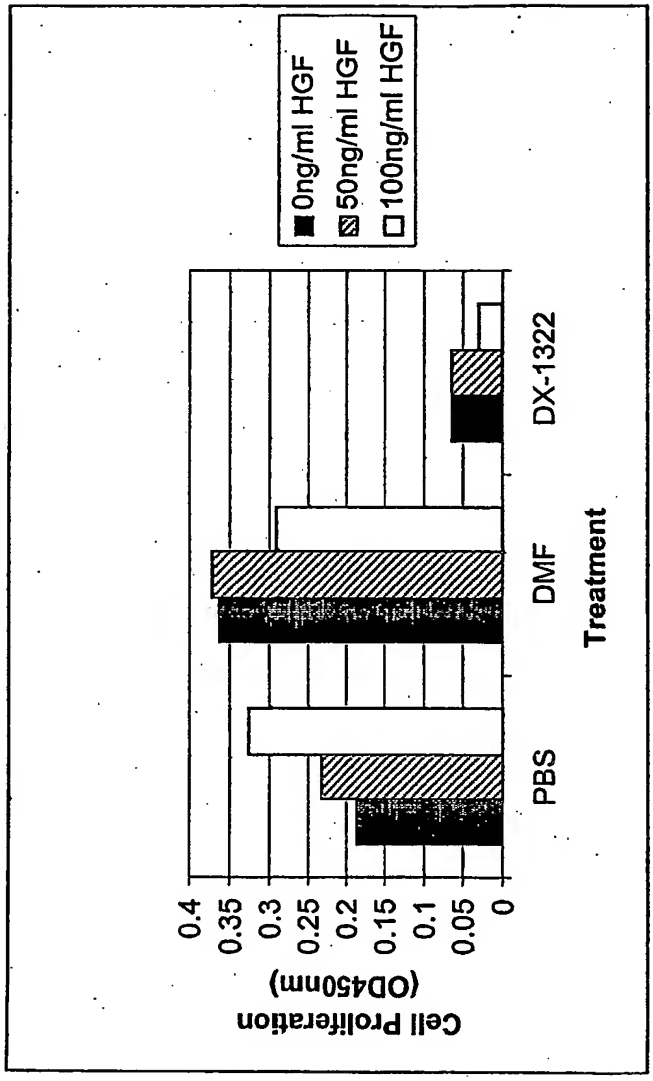

0ng/ml HGF
50ng/ml HGF
100ng/ml HGF
Cell Proliferation
(OD450nm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
PBS
DMF
DX-1322
Treatment

FIG. 11

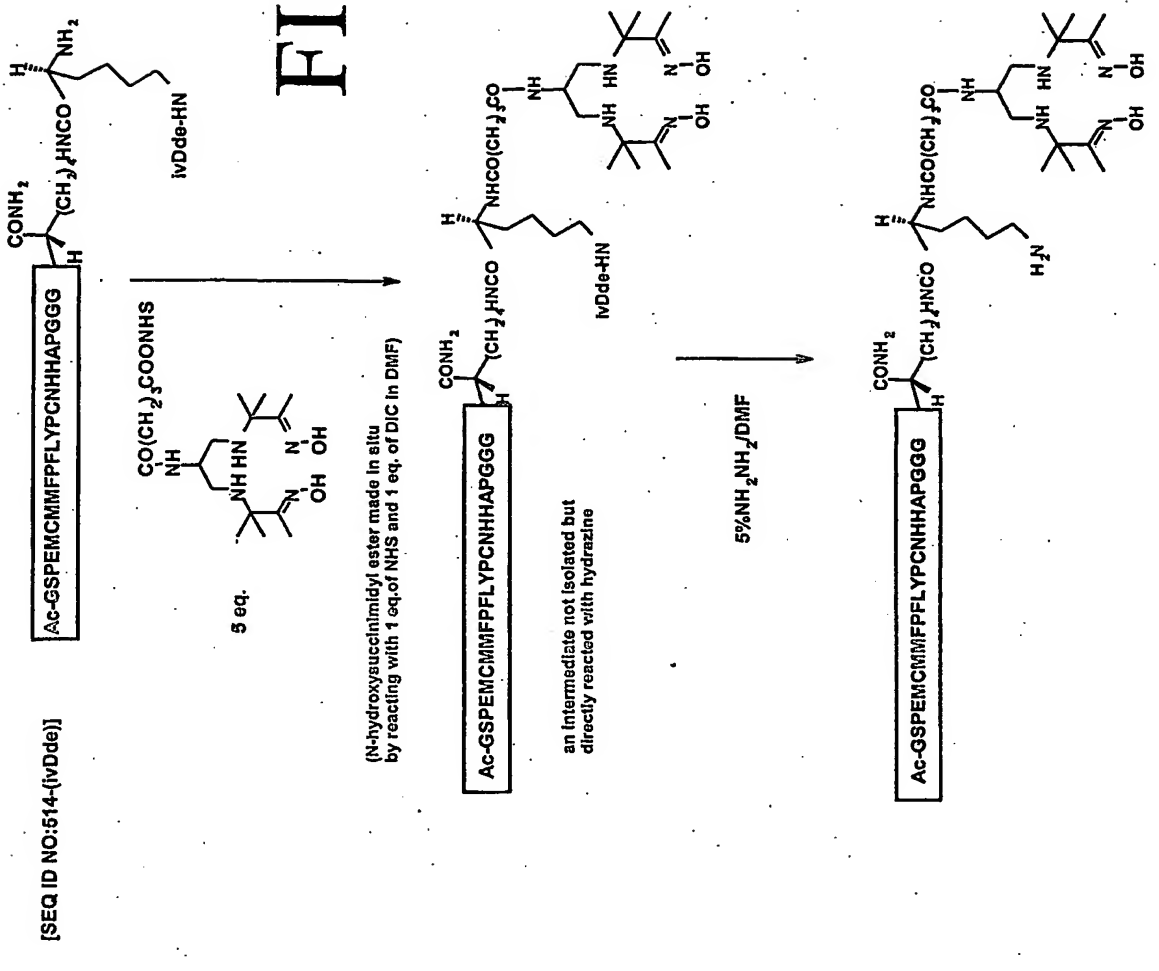
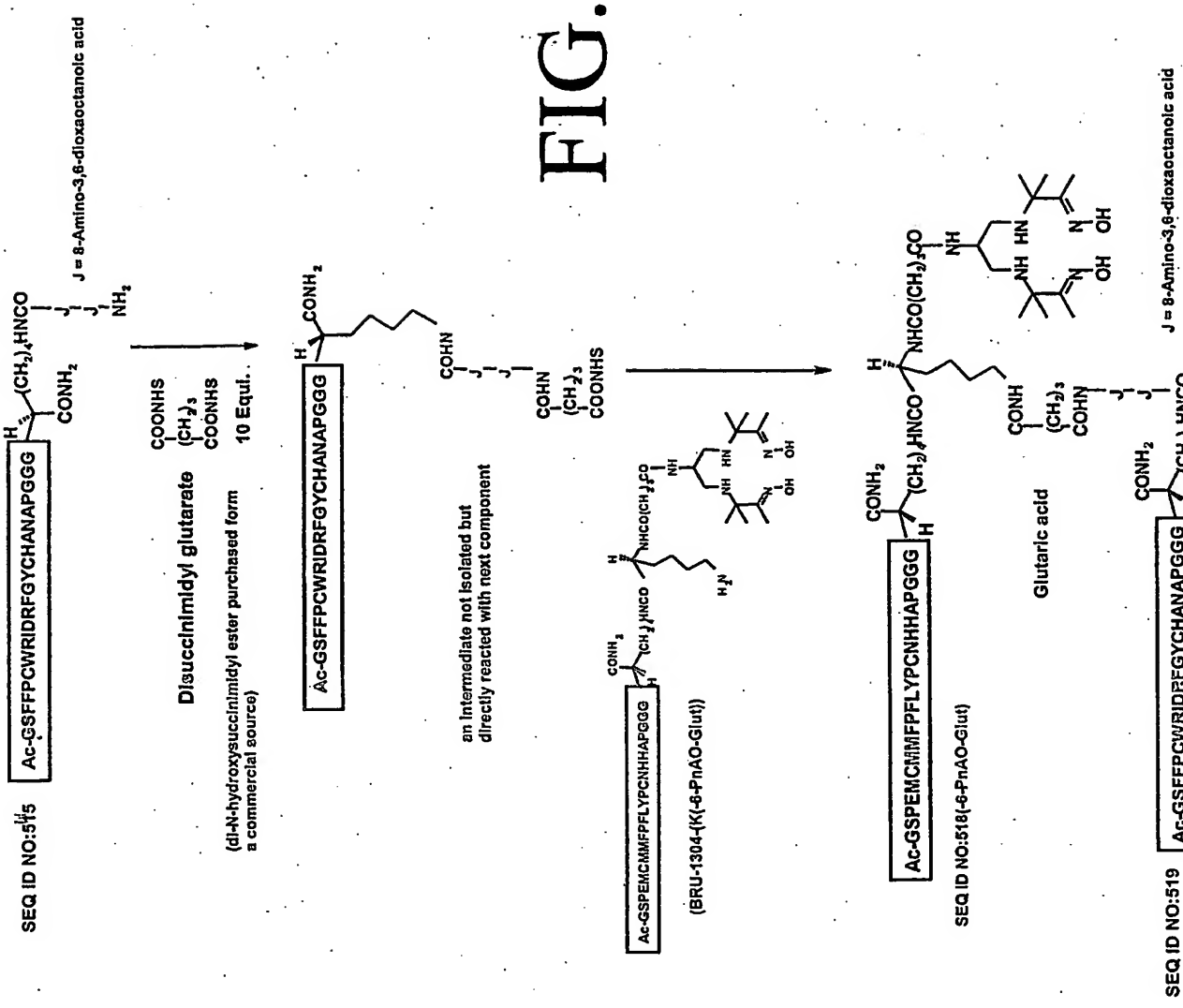

[SEQ ID NO:514-(ivDde)]
Ac-GSPEMCMMFPFLYPCNHHAPGGG
5 eq.
(N-hydroxysuccinimidyl ester made in situ by reacting with 1 eq.of NHS and 1 eq. of DIC in DMF)
an intermediate not isolated but directly reacted with hydrazine
5%NH2NH2/DMF

FIG. 12


SEQ ID NO:515
Ac-GSFFPCWRIDRFGYCHANAPGGG
J = 8-Amino-3,6-dioxaoctanoic acid
Disuccinimidyl glutarate
(di-N-hydroxysuccinimidyl ester purchased form a commercial source)
10 Equi.
Ac-GSFFPCWRIDRFGYCHANAPGGG
an Intermediate not isolated but directly reacted with next component
Ac-GSPEMCMMFPFLYPCNHHAPGGG
(BRU-1304-(K(-6-PnAO-Glut))
Ac-GSPEMCMMFPFLYPCNHHAPGGG
SEQ ID NO:518(-6-PnAO-Glut)
Glutaric acid
SEQ ID NO:519
Ac-GSFFPCWRIDRFGYCHANAPGGG
J = 8-Amino-3,6-dioxaoctanoic acid

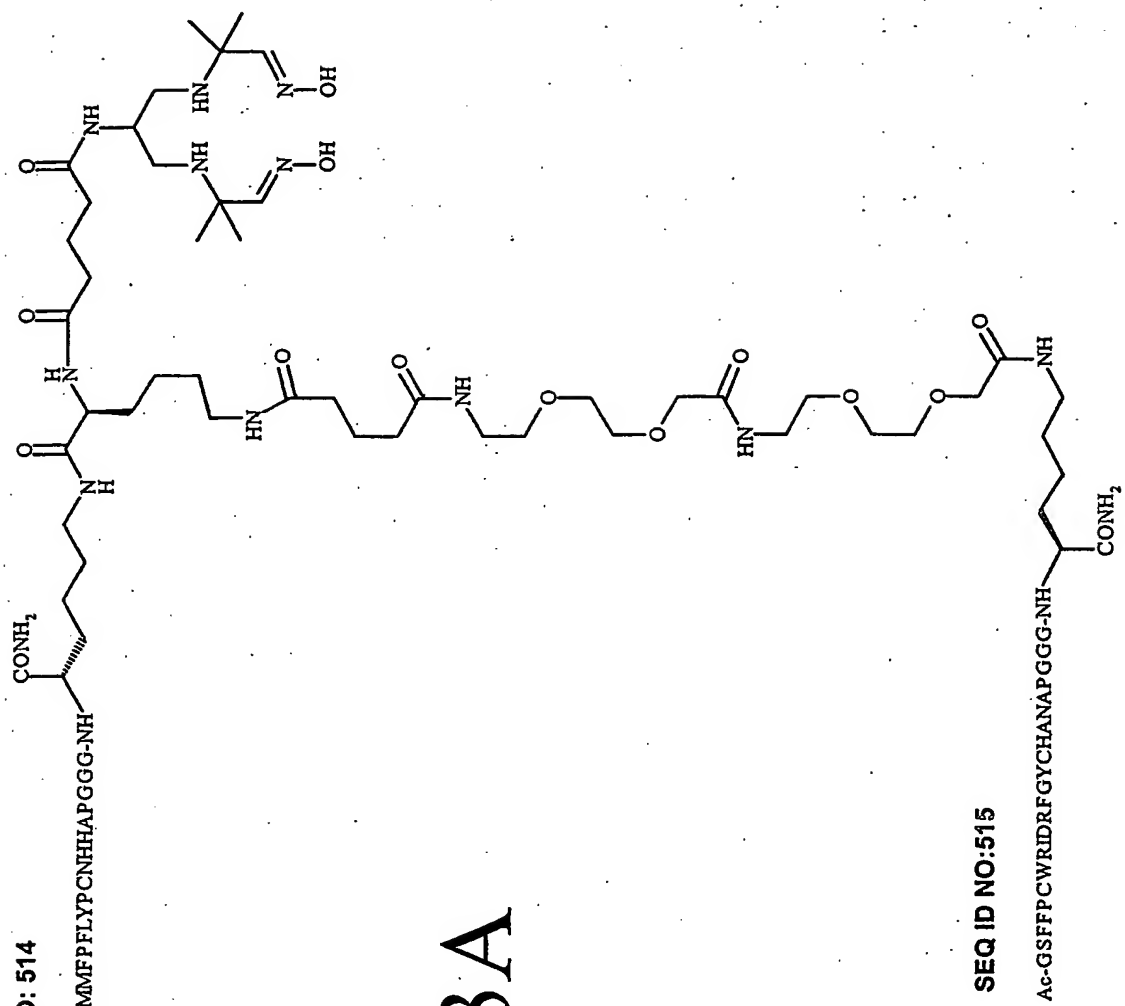

SEQ ID NO: 514
Ac-GSPEMCMMFPFLYPCNHHAPGGG-NH
SEQ ID NO:515
Ac-GSFFPCWRIDRFGYCHANAPGGG-NH

FIG. 13A

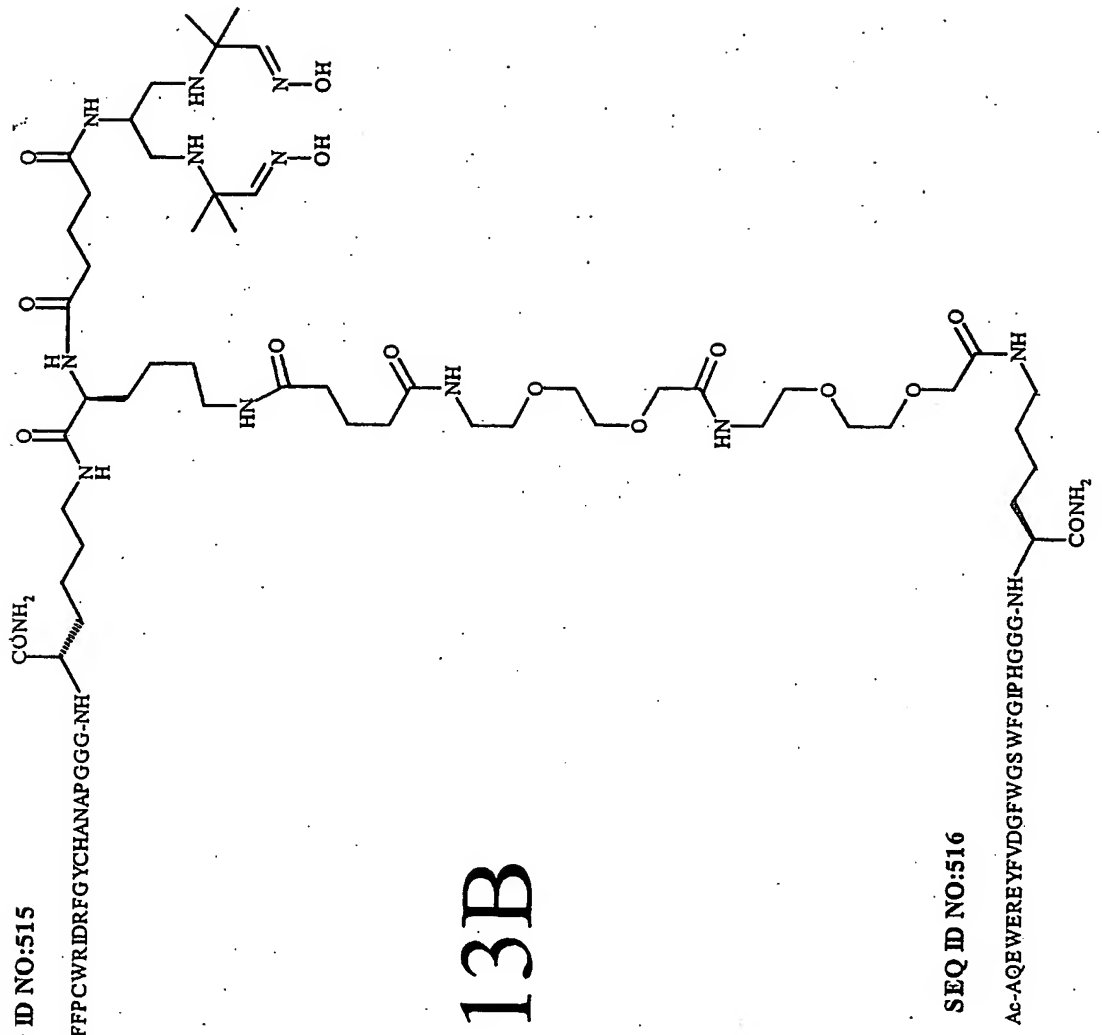

SEQ ID NO:515
Ac-GSFFPCWRIDRFGYCHANAPGGG-NH
SEQ ID NO:516
Ac-AQEWEREYFVDGFWGSWFGIPHGGG-NH
CONH2
CONH2

FIG. 13B

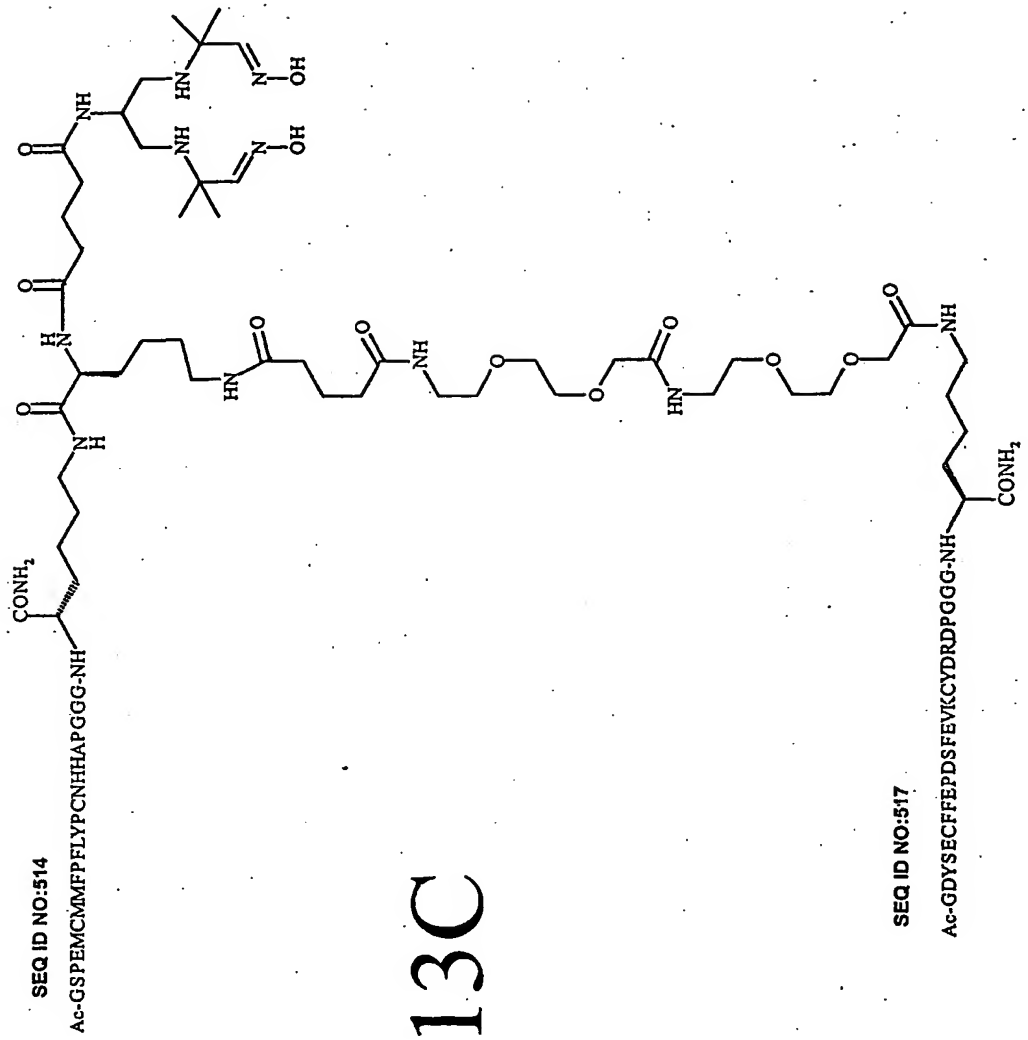
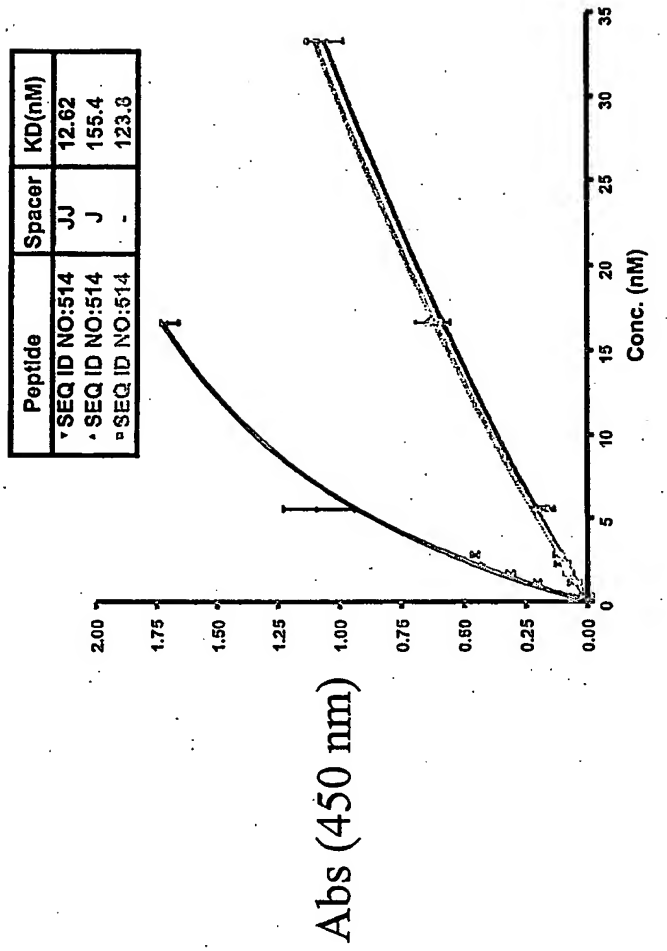

SEQ ID NO:514
Ac-GSPEMCMMFPFLYPCNHHAPGGG-NH
SEQ ID NO:517
Ac-GDYSECFFEPDSFEVKCYDRDPGGG-NH

FIG. 13C

FIG. 14


Peptide
Spacer
KD(nM)
▾SEQ ID NO:514
JJ
12.62
▴SEQ ID NO:514
J
155.4
▫SEQ ID NO:514
-
123.8
Abs (450 nm)
Conc. (nM)
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
0
5
10
15
20
25
30
35

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(71) Applicants *(for all designated States except US)*: **DYAX CORP.** [US/US]; 300 Technology Square, 8th Floor, Cambridge, MA 02139 (US). **BRACCO INTERNATIONAL B.V.** [NL/NL]; Stravinskylaan 3051, NL-1077 ZX Amsterdam (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SATO, Aaron, K.** [US/US]; 11 Pembroke Street, #2, Somerville, MA 02145 (US). **DRANSFIELD, Daniel, T.** [US/US]; 14 George Street, Hanson, MA 02341 (US). **LADNER, Robert, C.** [US/US]; 3827 Green Valley Road, Ijamsville, MD 21754 (US). **SHRIVASTAVA, Ajay** [IN/US]; 18-16 Pheasant Hollow Drive, Plainsboro, NJ 08536 (US). **NANJAPPAN, Palaniappa** [US/US]; 30 Liberty Drive, Dayton, NJ 08810 (US).

(74) Agent: **BUTLER LAWRENCE, Laurie**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

[Continued on next page]

(54) Title: PEPTIDES THAT SPECIFICALLY BIND HGF RECEPTOR (cMet) AND USES THEREOF

(57) Abstract: A polypeptide or multimeric polypeptide construct having the ability to bind to cMet or a complex comprising cMet and HGF, and methods for use are disclosed.

WO 2004/078778 A3

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/06473

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00
US CL : 514/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/14, 12, 2; 530/23.53; 424/143.1; 435/326, 69.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,871,959 A (RONG et al.) 16 February 1999 (16.02.1999), see entire document.	1, 25-31
A	US 2002/01136721 A1 (SCHWALL et al.) 26 September 2002 (26.09.2002), see entire document.	1, 25-31
A	US 2004/00118974 A1 (ARBOGAST et al.) 29 January 2004 (29.01.2004), see entire document.	1, 25-31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 July 2004 (14.07.2004)	07 OCT 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer B. Dell Chism Telephone No. (571) 272-1600

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US04/064[redacted]

Continuation of B. FIELDS SEARCHED Item 3:
STN BIOSIS MEDLINE SCISEARCH REGISTRY WEST
search terms:hepatocyte, cMet, tumor, antagonist, inhibitor

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/06473

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 2-24
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Theses claims 2-24 are drawn to subject matter that is not represented in the specification, i.e., amino acid sequences that are neither represented in a sequence listing nor in computer readable form.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.